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## (54) Mycosis vaccine

(57) The present invention pertains to vaccines comprising homogenised inactivated yeast blastospores and homogenised inactivated dermatophyte microconidia or antigenic material of said spores, methods for their production and their use for the prophylaxis and/or treatment of mycoses in mammals, preferably humans. The vaccines according to the present invention are especially useful for the prophylaxis and/or treatment of skin mycosis, preferably Dermatomycosis and/or Candidosis and/or Onychomycosis.

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## Description

The present invention is in the field of mycology and pertains to vaccines comprising homogenised inactivated dermatophyte microconidia and inactivated homogenised yeast blastospores or antigenic material of said spores, methods for their production and their use for the prophylaxis and/or treatment of mycoses in mammals, preferably humans. The vaccines according to the present invention are especially useful for the prophylaxis and/or treatment of skin mycoses, preferably Dermatomycosis and/or Candidosis and/or Onychomycosis.

Recently, the percentage of fungal infections (mycoses) has increased dramatically. Specifically, the percentage of fungal infections of the skin (skin mycoses) has increased to 4 - 8% of all skin diseases in humans. This percentage is increased up to 15 - 20% under tropical conditions. The most common pathogens associated with skin mycoses are dermatophytes of the genus Trichophyton, like Trichophyton rubrum, Trichophyton mentagrophytes and/or Trichophyton verrucosum. Other fungal pathogens associated with skin mycoses are yeasts, for example the genus Candida, i.e. Candida albicans.

A typical example for skin mycosis is Onychomycosis, i.e. Tinea unguium. Onychomycosis afflicts about 2 - 8% of the human population. Major pathogens associated with Onychomycosis in European countries are dermatophytes of the species Trichophyton rubrum and Trichophyton mentagrophytes as well as yeasts of the species Candida albicans. Candida albicans is found much more frequently in infected finger nails than toe nails. Unlike other skin mycoses, Onychomycoses never heal spontaneously and always lead in the terminal state to Onychodystrophy, if left untreated.

Skin mycoses are normally treated using a topical therapy with antimycotic chemical substances. However, these chemical substances have considerable side effects (e.g. hepatotoxicity, potential teratogenicity, gastrointestinal and central nervous system irritations as well as allergic reactions) and/or reach the target site only insufficiently, like in case of Onychomycosis, where the infected site is covered by the nail. Especially in chronic infections, where hair roots or nails are infected, these chemical therapies are lengthy and frustrating, for both the physician and the patient. Further, the rate of recurrence of infection is extremely high.

Skin mycoses can develop into systemic fungal infections (systemic mycosis), i.e. in immune compromised individuals. Systemic infections usually need to be treated with chemical agents for weeks or months. Treatment sometimes can last up to one year. Compliance of the patients often suffers when side-effects appear, and the benefit-risk-relation has become a special issue.

According to current knowledge, chronic fungal infections occur in otherwise healthy individuals, i.e. non immune deficient individuals, because in these individuals only an antibody response is triggered against the fungus, i.e. IgE mediated immunological response, but no cell-mediated immune response. However, the antibody-mediated immunological response alone is not sufficient to fight the fungus infection successfully. Chronic mycosis is the result (Sorensen, G.W., Arch. Dermatol. 112, 1976, 40-42; Hay, R.J., Shennan, G., Br. J. Dermatol. 106, 1982, 191-198; Dahl, M.V., Adv. Dermatol. 2, 1987, 305-320).

Vaccines comprising live dermatophytes are well known for their ability to elicit both responses, however, as with all live vaccine preparations, infection of healthy individuals by freshly vaccinated individuals is a permanent risk. Inactivated vaccines often fail to elicit a sufficient cell mediated response and accordingly are not as efficient as live vaccines.

Approaches concerning the use of inactivated dermatophytes as Dermatomycosis vaccines are known from prior art. For example Wharton, M. et al. (1950, J. Invest. Derm. 14, 291-303) teach active immunisation against Trichophyton purpureum infection in rabbits with an inactivated suspension of Trichophyton rubrum hyphae. EP 393371 and WO 9307894 teach inactivated Dermatomycosis vaccines comprising dermatophytes of the genus Trichophyton and/or Microsporum. To our knowledge, no mycoses vaccines are known from prior art, that comprise homogenised inactivated dermatophyte microconidia and inactivated homogenised yeast blastospores.

It was now surprisingly found, that vaccines comprising homogenised inactivated dermatophyte microconidia and inactivated homogenised yeast blastospores confer good resistance against fungal infections.

The present invention now provides vaccines comprising homogenised inactivated dermatophyte microconidia and inactivated homogenised yeast blastospores or antigenic material of said spores, methods for their production and their use for the prophylaxis and/or treatment of mycosis in mammals, preferably humans. The vaccines according to the present invention are especially useful for the prophylaxis and/or treatment of skin mycoses, preferably Dermatomycosis and/or Candidosis and/or Onychomycosis.

The vaccines of the present invention have excellent immunogenic properties in the absence of adverse side effects. In particular, the vaccines of the present invention do not provoke allergic reactions.

In one embodiment, the vaccines of the present invention comprise inactivated yeast blastospores and/or yeast blastospores that are in a swollen condition and/or have germ tubes and/or dermatophyte microconidia and/or dermatophyte microconidia that are in a swollen condition and/or have germ tubes, or antigenic material of said spores. Preferably, the yeast blastospores belong to the genus Candida, more preferably the species Candida albicans and/or the dermatophyte microconidia belong to the genus Trichophyton and/or Microsporum, i.e. the species Trichophyton

rubrum and/or Trichophyton mentagrophytes and/or Microsporum canis. Highly preferred are the strains Candida albicans DSM - 9456, and/or Candida albicans DSM - 9457 and/or Candida albicans DSM - 9458 and/or Candida albicans DSM - 9459 and/or Trichophyton rubrum DSM - 9469 and/or Trichophyton rubrum DSM - 9470 and/or Trichophyton rubrum DSM - 9471 and/or Trichophyton rubrum DSM - 9472 and/or Trichophyton mentagrophytes DSM - 7279 and/ or Microsporum canis DSM - 7281. Highly preferred are combinations of strains according to the examples. Preferably, 50% of the yeast blastospores and/or the dermatophyte microconidia are in swollen condition and/or have germ tubes. Preferably, the concentration of the spores is 40 to 90 million per ml, highly preferred is a concentration of about 60 million spores per ml. For inactivation of the spores, preferably thiomersal, formaldehyde or 2-propiolactone are used.

In another embodiment of the present invention, the yeast blastospores and/or dermatophyte microconidia are modified by chemical treatment, preferably by treatment with $H_2O_2$ and/or sodium permanganate and/or potassium permanganate.

The vaccines of the present invention can modulate the immune system, i.e. they have immunostimulatory properties and can be administered in the absence of additional immunostimmulatory substances. Therefore, in one embodiment, the vaccines of the present invention do not comprise adjuvants or other immunomodulatory or immunostimulatory substances.

To further increase their immunogenic properties, in another embodiment, the vaccines of the present invention further comprise at least one substance with immunomodulatory activity, preferably an adjuvant, preferably selected from the group of vitamin-E acetate, o/w-emulsion, aluminum phosphate, aluminium oxide, aluminium hydroxide/methyl cellulose gel, an oil-emulsion, muramil-dipeptides, Freund's adjuvants and saponins and/or at least one cytokine, preferably selected from the group of IL 2, IL 12, INF-Gamma.

In one embodiment, the vaccines of the present invention are used for the treatment and/or prophylaxis of mycoses, preferably skin mycosis, preferably Dermatomycosis and/or Candidosis and/or Onychomycosis in mammals, preferably humans.

In another embodiment, the vaccines of the present invention are used as immunomodulators, preferably immunostimulators.

The vaccines of the present invention can be administered parenterally, preferably by intramuscular injection and/or intraperitoneal injection and/or intracutaneous injection and/or percutaneous injection and/or topically, preferably cutaneously.

In another embodiment, the present invention provides processes for the preparation of the vaccines of the present invention. Said vaccines are preparable from dermatophytes and yeasts, preferably elected from the genera and/or species and/or strains indicated above, according to the following methods:

The first cultivation step for all of the below described processes is carried out according to the following:

Dermatophyte cultures, preferably of the genus Trichophyton and/or Microsporum, more preferably of the species Trichophyton mentagrophytes and/or Trichophyton rubrum or the strains Trichophyton rubrum DSM - 9469 and/or Trichophyton rubrum DSM - 9470 and/or Trichophyton rubrum DSM - 9471 and/or Trichophyton rubrum DSM - 9472 and/or Trichophyton mentagrophyes DSM - 7279 and/ or Microsporum canis DSM - 7281, are cultivated separately on agar/wort, for example in 3-10 Roux flasks. Each culture is cultivated for 15-30 days at 26-28°C.

Yeast cultures, preferably of the genus Candida, more preferably of the species Candida albicans or the strains Candida albicans DSM - 9456, and/or Candida albicans DSM - 9457 and/or Candida albicans DSM - 9458 and/or Candida albicans DSM - 9459, are cultivated separately in, for example 2-8 Roux flasks on agar Sabouraud or malt extract agar or other suitable media at 26-37°C for 1-7 days.

Fungal material obtainable according to this process is then preferably processed according to the following:

**Method 1 (exemplified in Examples 1 - 7)**

The fungal masses of the dermatophytes are lifted off and separately homogenised in an aqueous solution (for example 100-500 ml) of 0.1-0.3% fermented hydrolyzed muscle protein or 0.1-1% soy or pork peptone in combination with 5-6% glucose and 0.1-1% yeast extract. The concentration of microconidia is adjusted to 30-90 million per ml for each homogenate. Then each suspension of microconidia is fermented for 1-2 days at 28°C, to yield 50 to 100% germ tubes. After fermentation the cell suspensions can be washed with distilled water, physiological salt solution, for example sodium chloride or another suitable solution.

The blastospores of Candida albicans are washed off with a physiological solution of sodium chloride or another suitable solution. The concentration of blastospores in suspension is adjusted to 10-90 million per ml.

Equal volumes of each culture in suspension are mixed in a single container. The homogenate is inactivated by adding thiomersal in a ratio of 1:11000 to 1:25000 (w/v) directly to the cell suspension. The mixture is incubated for 1-3 days at room temperature.

The resulting vaccine is bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and can be stored refrigerated at 4-10°C. Vaccines preparable according to this method can be used for the prophylaxis and treatment of mycoses in mammals, preferably humans.

**Method 2 (exemplified in Examples 8 - 11)**

The fungal masses of the dermatophytes are lifted off and separately homogenised in an aqueous solution (for example 100-500 ml) of 0.1-0.3% fermented hydrolyzed muscle protein, 5-6 % glucose and 0.1-1% yeast extract. The concentration of microconidia is adjusted to 30-90 million per ml for each homogenate. To yield 50 to 100% germ tubes, each suspension of microconidia is fermented for 1-2 days at 28°C. Equal volumes of each dermatophyte culture in suspension are mixed in a single container. The homogenate is inactivated by adding thiomersal in a ratio of 1:10000 to 1:25000 (w/v) directly to the cell suspension. The mixture is incubated at room temperature for 1-2 days.

Each yeast culture is harvested and homogenised in 5000 ml medium RPMI No. 1640 comprising L-glutamine (Serva), medium No. 199 (Serva) or other suitable cell culture media. The concentration of the blastospores is adjusted to 10-30 million per ml. The fungi cell suspensions are incubated in cell culture flasks containing one of the above mentioned media in a $CO_2$ atmosphere of 5-6% at 36-38°C. After 2-4 hours incubation 50-100% of the blastospores commonly display germ tubes and a swollen condition. The blastospores are harvested and washed 3-5 times, for example by centrifugation (4000-6000 rpm) for 25-45 minutes for each centrifugation step at 4-10°C. Then the concentration of the cells is adjusted to 10-90 million per ml. The homogenate is inactivated by adding thiomersal in a ratio of 1:10000 to 1:25000 (w/v) directly to the cell suspension. The mixture is incubated at room temperature for 2 days. Equal volumes of each Candida albicans culture in suspension are mixed in a single container.

The dermatophyte and yeast cell suspensions are then mixed. The resulting vaccine is bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4-10°C. Vaccines preparable according to this method can be used for the prophylaxis and treatment of mycoses in mammals, preferably humans.

**Method 3 (exemplified in Examples 12 - 15)**

The fungal masses of the dermatophytes are lifted off and separately homogenised in an aqueous solution (for example 100-500 ml) of 0.1-0.3% fermented hydrolyzed muscle protein, 5-6% glucose and 0.1-1% yeast extract. The concentration of microconidia is adjusted to 30-90 million per ml for each homogenate. To yield 50-100% germ tubes, each suspension of microconidia is fermented for 1-2 days at 28°C.

The yeast blastospores are lifted off by washing with a physiological solution of sodium chloride or another suitable solution. The concentration of blastospores in suspension is adjusted to 10-90 million per ml.

Equal volumes of each culture in suspension are mixed in a single container. The homogenate is inactivated by adding thiomersal in a ratio of 1:11000 to 1:25000 (w/v) directly to the cell suspension. The mixture is then incubated at room temperature for 1-3 days.

Following the inactivation the cell suspension is treated with $H_2O_2$. For this purpose substances containing $H_2O_2$ are added to yield a final concentration of 1-3% of $H_2O_2$. The cell suspension is mixed for 14-48 hours. Treated cells are washed 3-5 times by centrifugation (4000 to 6000 rpm) for 20-50 minutes with destilled water or a physiological solution of sodium chloride. The final suspension of spores is adjusted to 30-90 million per ml.

Alternatively to $H_2O_2$ treatment, the cell suspensions can be treated with sodium or potassium permanganate. For this purpose a concentration of 1:10000 to 1:30000 (w/v) of sodium or potassium permanganate is added and the suspension is mixed for 10-48 hours. Treated cells are washed 3-5 times, for example with destilled water by centrifugation for 25-45 minutes for each centrifugation step (4000 rpm-6000 rpm). The final concentration of the spores is adjusted to 30-90 million per ml.

The dermatophyte and yeast cell suspensions are then mixed. The resulting vaccine is bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4-10°C. Vaccines preparable according to this method can be used for the prophylaxis and treatment of mycoses in mammals, preferably humans.

**Method 4 (exemplified in Examples 16 - 19)**

The fungal masses of the dermatophytes are lifted off and separately homogenised in an aqueous solution (for example 100-500 ml) of 0.1-0.3% fermented hydrolyzed muscle protein, 5-6% glucose and 0.1-1% yeast extract. The concentration of microconidia is adjusted to 30-90 million per ml for each homogenate. To yield 50-100% germ tubes each suspension of microconidia is fermented for 1-2 days at 28°C. Equal volumes of each dermatophyte culture in suspension are mixed in a single container. The homogenate is inactivated by adding thiomersal in a ratio of 1:10000 to

1:25000 (w/v) directly to the cell suspension. This mixture is incubated at room temperature for 1-2 days.

Following the inactivation, the cell suspension is treated with $H_2O_2$. For this purpose substances containing $H_2O_2$ are added to yield a final concentration of 1-3% of $H_2O_2$. The cell suspension is mixed for 14-48 hours. Treated cells are washed 3-5 times by centrifugation (4000 to 6000 rpm) for 20-50 minutes with destilled water or a physiological solution of sodium chloride. The final concentration of spores is adjusted to 30-90 million per ml.

Alternatively to $H_2O_2$ treatment, the cell suspensions can be treated with sodium or potassium permanganate. For this purpose a concentration of 1:10000 to 1:30000 (w/v) of sodium or potassium permanganate is added and the suspension is mixed for 10-48 hours. Treated cells are washed 3-5 times, for example with destilled water by centrifugation for 25-45 minutes for each centrifugation step (4000 rpm-6000 rpm). The final concentration of the spores is adjusted to 30-90 million per ml.

Each yeast culture is harvested and homogenised in 5000 ml medium RPMI No. 1640 comprising L-glutamine (Serva), medium No. 199 (Serva) or other types of medium for cell cultures. The concentration of the blastospores is adjusted to 10-30 million per ml. The fungi cell suspensions are incubated in cell culture flasks containing one of the above mentioned media in a $CO_2$ atmosphere of 5-6% at 36-38°C. After 2-4 hours incubation 50-100% of the blastospores commonly display germ tubes and a swollen condition. The blastospores are harvested and washed for 3-5 times by centrifugation (4000-6000 rpm) for 25-45 minutes at 4-10° C. The concentration of the spores is adjusted to 10-90 million per ml. The homogenate is inactivated by adding thiomersal in a ratio of 1:10000 to 1:25000 (w/v) directly to the cell suspension. The mixture is incubated at room temperature for 2 days.

Following the inactivation the cell suspension is treated with $H_2O_2$. For this purpose substances containing $H_2O_2$ are added to yield a final concentration of 1-3% of $H_2O_2$. Then the cell suspension is mixed for 14-48 hours. Treated cells are washed 3-5 times by centrifugation (4000-6000 rpm) for 20-50 minutes with destilled water or a physiological solution of sodium chloride. The final concentration of spores is adjusted to 30-90 million per ml.

Alternatively to $H_2O_2$ treatment, the cell suspensions can be treated with sodium or potassium permanganate. For this purpose a concentration of 1:10000 to 1:30000 (w/v) of sodium or potassium permanganate is added and the suspension is mixed for 10-48 hours. Treated cells are washed 3-5 times by centrifugation (4000-6000 rpm) for 25-45 minutes with destilled water. The final concentration of spores is adjusted to 30-90 million per ml. Then equal volumes of each yeast culture in suspension are mixed in a single container. The dermatophyte and yeast cell suspensions are then mixed. The resulting vaccine is bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4-10°C. Vaccines preparable according to this method can be used for the prophylaxis and treatment of mycoses in mammals, preferably humans.

**Method 5 (exemplified in Examples 20 - 23)**

The fungal masses of the dermatophytes are lifted off and separately homogenised in an aqueous solution (for example 100-500 ml) of 0.1-0.3% fermented hydrolyzed muscle protein, 5-6% glucose and 0.1-1% yeast extract. The concentration of microconidia is adjusted to 30-90 million per ml for each homogenate.

The yeast blastospores are lifted off by washing with a physiological solution of sodium chloride or another suitable solution. The concentration of blastospores in suspension is adjusted to 10-90 million per ml.

Equal volumes of each culture in suspension are combined and mixed in a single container. The homogenate is inactivated by adding thiomersal in a ratio of 1:11000 to 1:25000 (w/v) directly to the cell suspension. The mixture is incubated at room temperature for 1-3 days.

The resulting vaccine is bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4-10°C. Vaccines preparable according to this method can be used for the prophylaxis and treatment of mycoses in mammals, preferably humans.

**Method 6 (exemplified in examples 24-27)**

The fungal masses of the dermatophytes are lifted off and separately homogenised in an aqueous solution (for example 100-500 ml) of 0.1-0.3% fermented hydrolyzed muscle protein, 5-6% glucose and 0.1-1% yeast extract. The concentration of microconidia is adjusted to 30-90 million per ml for each homogenate.

The yeast blastospores are lifted off by washing with a physiological solution of sodium chloride or another suitable solution. The concentration of blastospores in suspension is adjusted to 10-90 million per ml.

Equal volumes of each culture in suspension are combined and mixed in a single container. The homogenate is inactivated by adding thiomersal in a ratio of 1:11000 to 1:25000 (w/v) directly to the cell suspension. The mixture is then incubated at room temperature for 1-3 days.

Following the inactivation, the cell suspension is treated with $H_2O_2$. For this purpose substances containing $H_2O_2$ are added to yield a final concentration of 1-3% of $H_2O_2$. Then the cell suspension is mixed for 14-48 hours. Treated cells are washed 3-5 times by centrifugation (4000-6000 rpm) for 20-50 minutes for each centrifugation step with des-

tilled water or a physiological solution of sodium chloride. The final concentration of cells is adjusted to 30-90 million per ml.

Alternatively to $H_2O_2$ treatment, the cell suspensions can be treated with sodium or potassium permanganate. For this purpose a concentration of 1:10000 to 1:30000 (w/v) of sodium or potassium permanganate is added and the suspension is mixed for 10-48 hours. Treated cells are washed 3-5 times, for example with destilled water by centrifugation for 25-45 minutes for each centrifugation step (4000-6000 rpm). The final concentration of the spores is adjusted to 30-90 million per ml.

The dermatophyte and yeast cell suspensions are then mixed. The resulting vaccine is bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4-10°C. Vaccines preparable according to this method can be used for the prophylaxis and treatment of mycosis in mammals, preferably humans.

The vaccines preparable according to methods 1 to 6, can be combined with a carrier, comprising a substance with immunomodulatory activity, preferably an adjuvant, preferably selected from the group of vitamin-E acetate, o/w-emulsion, aluminium phosphate, aluminium oxide, aluminium hydroxide/methyl cellulose gel, an oil-emulsion, muramil-dipeptides, Freund's adjuvants and saponins and/or at least one cytokine, preferably selected from the group of IL 2, IL 12, INF-Gamma, to futher increase the immunogenic activity of the vaccines of the present invention.

**1. Process for the preparation of an increased number of swollen microconidia and microconidia with germ tubes of dermatophytes**

Dermatophyte cultures are grown for 15-20 days in Roux flasks on solid agar surfaces (malt extract-agar, agar Sabouraud). The cultures are lifted off and homogenised with a sterile liquid medium of, for example, 0.3-1.0% crude extract or peptone from meat or soya, containing 5-6% glucose and 0.1-1.0% yeast extract or malt-extract broth or meat-glucose broth or others. The pH of the medium is maintained at 6.2-7.2. The concentration of microconidia in the fungal suspension is adjusted to 30-90 million per ml. For the second cultivation step (deep cultivation) the spore suspension is placed in a separate vessel containing the medium mentioned above. The deep cultivation is accomplished in 10-48 hours. 10-15 hours after beginning the cultivation, microscopical controls of the cell suspensions are made in order to count the number of swollen and germinated cells. Such controls are repeated every 5-6 hours. The cultivation is stopped when no less than 50% of the microconidia display a swollen or germinating condition and no more than 7-10% of the cells display a second mycelial branch. The diameter of swollen and germinated microconidia increases by 1.2 or more compared to regular microconidia.

**2. Process for the preparation of an increased number of swollen blastospores and blastospores with germ tubes of yeast**

Yeast cultures, preferably of Candida species are cultivated for 2-3 days on solid agar surfaces (malt extract-agar, agar Sabouraud). The cultures are lifted off and homogenised with a sterile liquid medium, preferably, medium No. 1640 (Serva) or medium No. 199 (Serva) or 0.3-1.0% meat extract comprising 5-6% glucose and 0.1-1.0% yeast extract adjusted to pH 6.8-7.0. The concentration of blastospores of the fungal suspension is adjusted to 1-20 million per ml. The resulting spore suspension is then placed in cell culture flasks or Petri dishes (2-5 mm height of liquid layer) and incubated in a $CO_2$ atmosphere of 5-6% at 36-38°C for 2-4 hours. The incubation process is stopped when 50% or more of the cells display germ tubes or a swollen condition. Swollen and germinated blastospores preparable according to this process have an increased diameter of 1.2 or more compared to regular blastospores.

In another embodiment the present invention provides highly immunogenic fungus strains as described below. These strains are especially suitable for the production of the highly immunogenic vaccines according to the present invention. All strains have been deposited by the applicant according to the Budapest Treaty at the 'Deutsche Sammlung von Mikroorganismen und Zellkulturen' (DSM), Mascheroder Weg 1B, W-38124 Braunschweig, Germany.

<u>TRICHOPHYTON RUBRUM, No. DSM- 9469</u>

The strain was deposited at the DSM on 26.10.1994 under Serial No. DSM-9469.

The strain was obtained by directed selection based on spore production and attenuation of the epizootic strain No. 533, which was identified on a skin of man in 1985. The strain was identified using the "Rebell-Taplin" key (Rebell, G., Taplin, D.: Dermatophytes, their recognition and identification, 3rd Print, University of Miami Press. Coral Gables, Florida, USA, 1978).

The biological properties of the strain are described in Table A.

Strain No. DSM-9469 differs from the epidemic strain in its faster growth in nutrient medium, an enormous production of microconidiae and lower virulence.

**TRICHOPHYTON RUBRUM, No. DSM- 9470**

The strain was deposited at the DSM on 26.10.1994 under Serial No. DSM-9470.

The strain was obtained by directed selection based on spore production and attenuation of the epizootic strain No. 535, which was identified on a skin of man in 1990. The strain was identified using the "Rebell-Taplin" key (Rebell, G., Taplin, D.: Dermatophytes, their recognition and identification, 3rd Print, University of Miami Press. Coral Gables, Florida, USA, 1978).

The biological properties of the strain are described in Table B. Strain No. DSM-9470 differs from the epidemic strain in its faster growth in nutrient medium, an enormous production of microconidiae and lower virulence.

**TRICHOPHYTON RUBRUM, No. DSM- 9471**

The strain was deposited at the DSM on 26.10.1994 under Serial No. DSM-9471.

The strain was obtained by directed selection based on spore production and attenuation of the epizootic strain No. 620, which was identified on a nail of man in 1989. The strain was identified using the "Rebell-Taplin" key (Rebell, G., Taplin, D.: Dermatophytes, their recognition and identification, 3rd Print, University of Miami Press. Coral Gables, Florida, USA, 1978).

The biological properties of the strain are described in Table C.

Strain No. DSM-9471 differs from the epidemic strain in its faster growth in nutrient medium, an enormous production of microconidiae and lower virulence.

**TRICHOPHYTON RUBRUM, No. DSM- 9472**

The strain was deposited at the DSM on 26.10.1994 under Serial No. DSM-9472.

The strain was obtained by directed selection based on spore production and attenuation of the epizootic strain No. 754, which was identified on a nail of man in 1990. The strain was identified using the "Rebell-Taplin" key (Rebell, G., Taplin, D.: Dermatophytes, their recognition and identification, 3rd Print, University of Miami Press. Coral Gables, Florida, USA, 1978).

The biological properties of the strain are described in Table D.

Strain No. DSM-9472 differs from the epidemic strain in its faster growth in nutrient medium, an enormous production of microconidiae and lower virulence.

**CANDIDA ALBICANS, No. DSM- 9456**

The strain was deposited at the DSM on 26.10.1994 under Serial No. DSM-9456.

The strain was obtained by directed selection based on stabilisation of cultural-morphological characteristics and attenuation of epidemic strain No. 008-L, which was identified on man in 1990. The strain was identified using the Lodder's key (Lodder,J: The yeast: A Taxonomic Study. North-Holland Publ. Co., Amsterdam - London (1970).

The biological properties of the strain are described in Table E.

Strain No. DSM-9456 differs from the epidemic strain in its faster growth in nutrient medium, stabile biological properties, an enormous production of biomass and lower virulence.

**CANDIDA ALBICANS, No. DSM- 9457**

The strain was deposited at the DSM on 26.10.1994 under Serial No. DSM-9457.

The strain was obtained by directed selection based on stabilisation of cultural-morphological characteristics and attenuation of epidemic strain No. 012, which was identified on man in 1992. The strain was identified using the Lodder's key (Lodder,J: The yeast: A Taxonomic Study. North-Holland Publ. Co., Amsterdam - London (1970).

The biological properties of the strain are described in Table F.

Strain No. DSM-9457 differs from the epidemic strain in its faster growth in nutrient medium, stabile biological properties, an enormous production of biomassand lower virulence.

**CANDIDA ALBICANS, No. DSM- 9458**

The strain was deposited at the DSM on 26.10.1994 under Serial No. DSM-9458.

The strain was obtained by directed selection based on stabilisation of cultural-morphological characteristics and attenuation of epidemic strain No. 047, which was identified on man in 1989. The strain was identified using the Lodder's key (Lodder,J: The yeast: A Taxonomic Study. North-Holland Publ. Co., Amsterdam - London (1970).

The biological properties of the strain are described in Table G.

Strain No. DSM-9458 differs from the epidemic strain in its faster growth in nutrient medium, stabile biological properties, an enormous production of biomass and lower virulence.

CANDIDA ALBICANS, No. DSM- 9459

The strain was deposited at the DSM on 26.10.1994 under Serial No. DSM-9459.

The strain was obtained by directed selection based on stabilisation of cultural-morphological characteristics and attenuation of epidemic strain No. 158, which was identified on man in 1990. The strain was identified using the Lodder's key (Lodder,J: The yeast: A Taxonomic Study. North-Holland Publ. Co., Amsterdam - London (1970).

The biological properties of the strain are described in Table H.

Strain No. DSM-9459 differs from the epidemic strain in its faster growth in nutrient medium, stable biological properties, an enormous production of biomass and lower virulence.

Strains Trichophyton mentagrophytes DSM-7279 and Microsporum canis DSM-7281 have been deposited at the DSM by the applicant on 01.10.1992 under the Budapest Treaty and are described for example in applicant's Patent Application No. PCT/EP92/02391, published as WO 93/07894 on 29.04.1993.

TABLE A

| Properties and characteristics of the strains | Strain No. DSM-9469 | Epidemic Strain No. 533 |
|---|---|---|
| Description of the culture | Mature 15-day colony on agar Sabouraud: white, velvety, flat, margin of the colony fringed, under surface yellow, in centre deep purple, diameter of colony 60 - 63 mm | 20-day colony on agar Sabouraud: white, downy, elevated, margin of colony regular, under surface purple, diameter of colony 30 - 35 mm |
| Morphological characteristics | Mature 15-day culture with septate branching hyphae 1 - 3 µm wide, numerous obovate oval microconidia measuring 2 -3 x 3 - 5 µm, macroconidia long clavate pencil-shaped with 4-5 cross walls measuring 4 - 6 x 15 - 40 µm. | 20-day culture with septate branching hyphae 1 - 3 µm wide, microconidia clavate to round in small open clusters and along the hyphae measuring 2 - 3 x 3 - 6 µm; macroconidia are rare, long and pencil-shaped with 3 - 5 cross walls measuring 4 - 7 x 15 - 50 µm. |
| Pathogenic characteristics | The strain is weakly virulent. 9 -10 days after application of a dose of 500-600 thousand cells of fungal material per $cm^2$ on scarified skin of guinea pigs, scales are formed. Spontaneous recovery after 18 - 20 days. | The strain is virulent. 9 - 10 days after application of a dose of 500-600 thousand cells of fungal material per $cm^2$ on scarified skin of guinea pigs, thin necrotic scabs are formed. Spontaneous recovery after 25 - 30 days. |
| Reaction response | Result of intramuscular injection of inactivated corpuscular antigens from cultures: no observed changes in clinical state of animals | Result of intramuscular injection of inactivated corpuscular antigens from cultures: inflammation at point of injection, oedema |
| Immunogenic response | Results of immunisation of a group of guinea pigs with inactivated antigen from cultures (repeated not less than 5 times): establishes immunity | Results of immunisation of a group of guinea pigs with inactivated antigen from cultures (repeated not less than 5 times): establishes immunity |

TABLE B

| Properties and characteristics of the strains | Strain No. DSM-9470 | Epidemic Strain No. 535 |
|---|---|---|
| Description of the culture | Mature 15-day colony on agar Sabouraud: white velvety-fluffy in centre, folded, margin of colony regular, under surface colourless or rose, diameter of colony 25 - 30 mm | 20-day colony on agar Sabouraud: white, fluffy, margin of colony regular, under surface yellow, 20 mm in diameter |
| Morphological characteristics | Mature 15-day culture with septate branching hyphae 1 - 3 μm wide, round oval puriform microconidia measuring 2 - 3 x 3 - 7 μm. | 20-day culture with septate branching hyphae 1 - 3 μm wide, microconidia clavate to round in small open clusters and along the hyphae measuring 2 - 3 x 3 - 6 μm; macroconidia are absent. |
| Pathogenic characteristics | The strain is weakly virulent. 9-10 days after application of a dose of 500 - 600 thousand cells of fungal material per $cm^2$ on scarified skin of guinea pigs, necrotic scabs are formed. Spontaneous recovery after 22 - 25 days. | The strain is virulent. 9-10 days after application of a dose of 500-600 thousand cells of fungal material per $cm^2$ on scarified skin of guinea pigs, thin necrotic scabs are formed. Spontaneous recovery after 25 - 30 days. |
| Reaction response | Result of intramuscular injection of inactivated corpuscular antigens from cultures: no observed changes in clinical state of animals | Result of intramuscular injection of inactivated corpuscular antigens from cultures: inflammation at point of injection, oedema |
| Immunogenic response | Results of immunisation of a group of guinea pigs with inactivated antigen from cultures (repeated not less than 5 times): establishes immunity | Results of immunisation of a group of guinea pigs with inactivated antigen from cultures (repeated not less than 5 times): establishes immunity |

TABLE C

| Properties and characteristics of the strains | Strain No. DSM-9471 | Epidemic Strain No. 620 |
|---|---|---|
| Description of the culture | Mature 15-day colony on agar Sabouraud: white, velvety, elevated, margin of colony regular, under surface yellow, in centre deep purple, diameter of colony 32 - 35 mm | 20-day colony on agar Sabouraud: white, downy, elevated, margin of colony regular, under surface purple, diameter of colony 20 - 25 mm |
| Morphological characteristics | Mature 15-day culture with septate branching hyphae 1 -3 μm wide, round oval puriform microconidia measuring 2 - 3 x 3 - 7 μm. | 20-day culture with septate branching hyphae 1 - 3 μm wide, microconidia clavate to round in small open clusters and along the hyphae measuring 2 - 3 x 3 - 6 μm; macroconidia are rare, long and pencil-shaped with 3 - 5 cross walls measuring 4 - 7 x 15 - 50 μm. |
| Pathogenic characteristics | The strain is weakly virulent. 9 - 10 days after application of a dose of 500 - 600 thousand cells of fungal materials per $cm^2$ on scarified skin of guinea pigs, scales are formed. Spontaneous recovery after 18 - 20 days. | The strain is virulent. 9 - 10 days after application of a dose of 500 -600 thousand cells of fungal materials per $cm^2$ on scarified skin of guinea pigs, thin necrotic scabs are formed. Spontaneous recovery after 25 - 30 days. |
| Reaction response | Result of intramuscular injection of inactivated corpuscular antigens from cultures: no observed changes in clinical state of animals | Result of intramuscular injection of inactivated corpuscular antigens from cultures: inflammation at point of injection, oedema |
| Immunogenic response | Results of immunisation of a group of guinea pigs with inactivated antigen from cultures (repeated not less than 5 times): establishes immunity | Results of immunisation of a group of guinea pigs with inactivated antigen from cultures (repeated not less than 5 times): establishes immunity |

TABLE D

| Properties and characteristics of the strains | Strain No. DSM-9472 | Epidemic Strain No. 754 |
|---|---|---|
| Description of the culture | Mature 15-day colony on agar Sabouraud: white, velvety, in centre folded, margin of colony regular, under surface yellow in centre purple, diameter of colony 35 - 40 mm | 20-day colony on agar Sabouraud: white-rose, downy, margin of colony regular, under surface purple, diameter of colony 20 - 25 mm |
| Morphological characteristics | Mature 15-day culture with septate branching hyphae 1 -3 μm wide, round oval puriform microconidia measuring 2 - 3 x 3 - 7 μm. | 20-day culture with septate branching hyphae 1-3 μm wide, microconidia clavate to round in small open clusters and along the hyphae measuring 2 - 3 x 3 - 6 μ m; macroconidia are rare, long and pencil-shaped with 3 - 5 cross walls measuring 4 - 7 x 15 - 50 μm. |
| Pathogenic characteristics | The strain is weakly virulent. 9 -10 days after application of a dose of 500-600 thousand cells of fungal materials per $cm^2$ on scarified skin of guinea pigs, scales are formed. Spontaneous recovery after 18 - 20 days. | The strain is virulent. 9 - 10 days after application of a dose of 500 -600 thousand cells of fungal materials per $cm^2$ on scarified skin of guinea pigs, thin necrotic scabs are formed. Spontaneous recovery after 25 - 30 days. |
| Reaction response | Result of intramuscular injection of inactivated corpuscular antigens from cultures: no observed changes in clinical state of animals | Result of intramuscular injection of inactivated corpuscular antigens from cultures: inflammation at point of injection, oedema |
| Immunogenic response | Results of immunisation of a group of guinea pigs with inactivated antigen from cultures (repeated not less than 5 times): establishes immunity | Results of immunisation of a group of guinea pigs with inactivated antigen from cultures (repeated not less than 5 times): establishes immunity |

TABLE E

| Properties and characteristics of the strains | Strain No. DSM-9456 | Epidemic Strain No. 008-L |
|---|---|---|
| Description of the culture | 10-day single-spore colony on agar Sabouraud: cream smooth and pasty glistening, elevated, margin of colony regular, diameter of colony 20 - 30 mm | 10-day single-spore colony on agar Sabouraud: cream soft and smooth with feathery offshots at the edges, diameter of colony 10 -15 mm |
| Morphological characteristics | 10-day culture with spherical oval blastospores measuring 3.5 - 6 x 6 - 10 μm, chlamidospores 12 - 15 μm wide, pseudohyphae 5 - 8 μ m wide, hyphae 1.5 - 3 μm wide | 10-day single-spore culture on agar Sabouraud with spherical oval budding blastospores measuring 3 -5 x 5 -8 μm, chlamidospores 10 - 15 μm diameter, pseudohyphae 5 - 8 μm wide, hyphae 1.5 - 3 μm wide. |
| Pathogenic characteristics | The strain is weakly virulent. 30 days after intraperitoneal injection of a dose of 10 -100 million fungal cells to white mice, granuloma in abdominal organs of 50% of animals are formed. Lethal effect was not observed. | The strain is weakly virulent. 30 days after intraperitoneal injection of a dose of 10 - 100 million fungal cells to white mice, granuloma in abdominal organs of 80 - 100% of animals are formed. Lethal effect in 50 - 70% was observed. |
| Reaction response | Result of intramuscular injection of inactivated corpuscular antigens from cultures: no observed changes in clinical state of animals | Result of intramuscular injection of inactivated corpuscular antigens from cultures: inflammation at point of injection, oedema |
| Immunogenic response | Results of immunisation of a group of white mice with inactivated antigen from cultures (repeated not less than 10 times): establishes immunity | Results of immunisation of a group of white mice with inactivated antigen from cultures (repeated not less than 10 times): establishes immunity |

TABLE F

| Properties and characteristics of the strains | Strain No DSM-9457 | Epidemic Strain No. 012 |
|---|---|---|
| Description of the culture | 10-day single-spore colony on agar Sabouraud: cream rough elevated, margin of colony lobulated, diameter of colon 20 - 23 mm | 10-day single-spore colony on agar Sabouraud: cream rough elevated, margin of colony fringed and lobulated, diameter of colony 15 - 20 mm |
| Morphological characteristics | 10-day single-spore culture with spherical oval blastospores measuring 3.5 - 5 x 5 - 10 μm, chlamidospores 12 - 15 μm wide, pseudohyphae 4 - 7 μ m wide, hyphae 2 - 3 μm wide | 10-day single-spore culture on agar Sabouraud with spherical oval budding blastospores measuring 3 - 5 x 5 -8 μm, chlamidospores 10 - 15 μm diameter, pseudohyphae 5 - 8 μm wide, hyphae - 1.5 - 3 μm wide |
| Pathogenic characteristics | The strain is weakly virulent. 30 days after intraperitoneal injection of a dose of 10 -100 million fungal cells to white mice, granuloma in abdominal organs in 30% of animals are formed. Lethal effect was not observed. | The strain is weakly virulent. 30 days after intraperitoneal injection by dose of 10 - 100 million fungal cells to white mice, granuloma in abdominal organs of 50% of animals are formed. Lethal effect not more 50 % were observed. |
| Reaction response | Result of intramuscular injection of inactivated corpuscular antigens from cultures: no observed changes in clinical state of animals | Result of intramuscular injection of inactivated corpuscular antigens from cultures: no observed changes in clinical state of animals |
| Immunogenic response | Results of immunisation of a group of white mice with inactivated antigen from cultures (repeated not less than 10 times): establishes immunity | Results of immunisation of a group of white mice with inactivated antigen from cultures (repeated not less than 10 times): establishes immunity |

TABLE G

| Properties and characteristics of the strains | Strain No. DSM-9458 | Epidemic Strain No. 047 |
|---|---|---|
| Description of the culture | 10-day single-spore colony on agar Sabouraud: cream smooth and pasty glistening, elevated, margin of colony regular, diameter of colony 16 - 18 mm | 10-day single-spore colony on agar Sabouraud: cream soft and smooth with feathery offshots at the edges, diameter of colony 10 -15 mm |
| Morphological characteristics | 10-day culture with spherical oval blastospores measuring 3.6 - 6 x 6 - 11 μm, chlamidospores 12 -15 μm wide, pseudohyphae 4 - 8 μ m wide, hyphae 1.5 - 3 μm wide | 10-day single-spore culture on agar Sabouraud with spherical oval budding blastospores measuring 3 - 5 x 5 - 8 μm, chlamidospores 10 - 15 μm diameter, pseudohyphae 5 - 8 μm wide, hyphae 1.5 - 3 μm wide. |
| Pathogenic characteristics | The strain is weakly virulent. 30 days after intraperitoneal injection of a dose of 10 -100 million fungal cells to white mice, granuloma in abdominal organs of 50 -100% of animals are formed. Lethal effect in 50% were observed. | The strain is weakly virulent. 30 days after intraperitoneal injection by dose of 10 - 100 million fungal cells to white mice, granuloma in abdominal organs of 80 - 100% of animals are formed. Lethal effect in 70 - 100 % were observed. |
| Reaction response | Result of intramuscular injection of inactivated corpuscular antigens from cultures: no observed changes in clinical state of animals | Result of intramuscular injection of inactivated corpuscular antigens from cultures: inflammation at point of injection, oedema |
| Immunogenic response | Results of immunisation of a group of white mice with inactivated antigen from cultures (repeated not less than 10 times): establishes immunity | Results of immunisation of a group of white mice with inactivated antigen from cultures (repeated not less than 10 times): establishes immunity |

TABLE H

| Properties and characteristics of the strains | Strain No. DSM-9459 | Epidemic Strain No. 158 |
|---|---|---|
| Description of the culture | 10-day single-spore colony on agar Sabouraud: cream smooth pasty glistening, elevated, margin of colony regular, diameter of colony 16 - 18 mm | 10-day single-spore colony on agar Sabouraud: cream smooth pasty, margin of colony lobulated and with feathery offshots at the edges, diameter of colony 10 - 15 mm |
| Morphological characteristics | 10-day culture with spherical oval blastospores measuring 3.6 - 6 x 6 - 11 μm, chlamidospores 12 - 15 μm wide, pseudohyphae 4 - 8 μ m wide, hyphae 1.5 - 3 μm wide | 10-day single-spore culture on agar Sabouraud with spherical oval budding blastospores measuring 3 - 5 x 5 -8 μm, chlamidospores 10 - 15 μm diameter, pseudohyphae 5 - 8 μm wide, hyphae 1.5 - 3 μm wide. |
| Pathogenic characteristics | The strain is weakly virulent. 30 days after intraperitoneal injection by dose of 10 -100 million fungal cells to white mice, granuloma in abdominal organs of 40% of animals are formed. Lethal effect was not observed. | The strain is weakly virulent. 30 days after intraperitoneal injection by dose of 10 -100 million fungal cells to white mice, granuloma in abdominal organs of 50% of animals are formed. Lethal effect in 20 - 50% was observed. |
| Reaction response | Result of intramuscular injection of inactivated corpuscular antigens from cultures: no observed changes in clinical state of animals | Result of intramuscular injection of inactivated corpuscular antigens from cultures: inflammation at point of injection, oedema |
| Immunogenic response | Results of immunisation of a group of white mice with inactivated antigen from cultures (repeated not less than 10 times): establishes immunity | Results of immunisation of a group of white mice with inactivated antigen from cultures (repeated not less than 10 times): establishes immunity |

**Brief Description of the Figures**

Figure 1. The dynamics of clinical symptoms of Trichophyton rubrum infections in guinea pigs (1st experiment). Compared with the severity score values of the control group the efficacy of Complex I-I was 100%, 36%, 40% and 100% after 7, 13, 21 and 28 days respectively. In guinea pigs vaccinated with Complex I-II, the severity score was diminished by 40%, 44%, 30% and 56% when compared with control values.

Figure 2. The dynamics of clinical symptoms of Trichophyton rubrum infections in guinea pigs (Complex I-I). Severity score values (mean) of vaccinated animals (Complex I-I) are compared with control values (mean) of unvaccinated animals after different observation periods. Efficacy is 100%, 34%, 40% and 100% after 7, 13, 21 and 28 days respectively.

Figure 3. The dynamics of clinical symptoms of Trichophyton rubrum infections in guinea pigs (Complex I-II). Severity score values (mean) of vaccinated animals (Complex I-II) are compared with control values (mean) of unvaccinated animals after different observation periods. Efficacy is 40%, 44%, 30% and 56% after 7, 13, 21 and 28 days respectively.

Figure 4. The dynamics of clinical symptoms of Trichophyton rubrum infections in guinea pigs (2nd experiment). Compared with the severity score values of the control group the efficacy of Complex II-I was 100%, 32.4%, 79.4% and 74.6% after 7, 15, 21 and 28 days respectively. In guinea pigs vaccinated with Complex II-II severity score was diminished by 84.4%, 34%, 56% and 42.5% when compared with control values.

Figure 5. The dynamics of clinical symptoms of Trichophyton rubrum infections in guinea pigs (Complex II-I). Severity score values (mean) of vaccinated animals (Complex II-I) are compared with control values (mean) of

unvaccinated animals after different observation periods. Efficacy is 100%, 32.4%, 79.4% and 74.6% after 7, 15, 21 and 28 days respectively.

Figure 6. The dynamics of clinical symptoms of Trichophyton rubrum infections in guinea pigs (Complex II-II). Severity score values (mean) of vaccinated animals (Complex II-II) are compared with control values (mean) of unvaccinated animals after different observation periods. Efficacy is 84.4%, 34%, 56% and 42.4% after 7, 15, 21 and 28 days respectively.

Figure 7. The dynamics of clinical symptoms of Trichophyton rubrum infections in guinea pigs (3rd experiment). Compared with the severity score values of the control group the efficacy of Complex II-I was 78.2%, 48%, 100% and 100%, Complex II-II 72.8%, 50%, 100% and 100%, Complex II-III 34.4%, 20%, 50% and 0%, Complex II-IV 10%, 20%, 44% and 37.5%, Complex II-V 34.5%, 36%, 35% and 20% after 7, 16, 21 and 28 days respectively. Note the low severity score value and fast healing process in animals vaccinated with Complexes II-I and II-II.

Figure 8. The dynamics of clinical symptoms of Trichophyton rubrum infections in guinea pigs (Complex III-I). Severity score values (mean) of vaccinated animals (Complex III-I) are compared with control values (mean) of unvaccinated animals after different observation periods. Efficacy is 78.2%, 48%, 100% and 100% after 7, 16, 21 and 28 days respectively.

Figure 9. The dynamics of clinical symptoms of Trichophyton rubrum infections in guinea pigs (Complex III-II). Severity score values (mean) of vaccinated animals (Complex III-II) are compared with control values (mean) of unvaccinated animals after different observation periods. Efficacy is 72.8%, 50%, 100% and 100% after 7, 16, 21 and 28 days respectively.

Figure 10. The dynamics of clinical symptoms of Trichophyton rubrum infections in guinea pigs (Complex III-III). Severity score values (mean) of vaccinated animals (Complex III-III) are compared with control values (mean) of unvaccinated animals after different observation periods. Efficacy is 34.4%, 20%, 50% and 0% after 7, 16, 21 and 28 days respectively.

Figure 11. The dynamics of clinical symptoms of Trichophyton rubrum infections in guinea pigs (Complex III-IV). Severity score values (mean) of vaccinated animals (Complex III-IV) are compared with control values (mean) of unvaccinated animals after different observation periods. Efficacy is 10%, 20%, 44% and 37.5% after 7, 16, 21 and 28 days respectively.

Figure 12. The dynamics of clinical symptoms of Trichophyton rubrum infections in guinea pigs(Complex III-V). Severity score values (mean) of vaccinated animals (Complex III-V) are compared with control values (mean) of unvaccinated animals after different observation periods. Efficacy is 34.5%, 36%, 35% and 20% after 7, 16, 21 and 28 days respectively.

Figure 13. The dynamics of clinical symptoms of Trichophyton mentagrophytes infections in guinea pigs (3rd experiment).
In guinea pigs vaccinated with complex III-I and III-II, severity scores of clinical symptoms when compared with values obtained from control animals were lower during the complete observation period. Guinea pigs vaccinated with complex III-III, III-IV or III-V had intensive symptoms of a Trichophyton mentagrophytes infection on days 7 and 16 but in comparison with control animals these symptoms were markedly reduced at the following observation dates .

Figure 14. The dynamics of clinical symptoms of Trichophyton mentagrophytes infections in guinea pigs(Complex III-I).
Severity score values (mean) of vaccinated animals (Complex III-I) are compared with control values (mean) of unvaccinated animals after different observation periods. Efficacy is 50%, 9.1%, 33.5% and 71.5% after 7, 16, 21 and 28 days respectively.

Figure 15. The dynamics of clinical symptoms of Trichophyton mentagrophytes infections in guinea pigs(Complex III-II).
Severity score values (mean) of vaccinated animals (Complex III-II) are compared with control values (mean) of unvaccinated animals after different observation periods. Efficacy is 55%, 13.6%, 33.3% and 69.3% after 7, 16, 21 and 28 days respectively.

Figure 16. The dynamics of clinical symptoms of Trichophyton mentagrophytes infections in guinea pigs (Complex III-III).
Severity score values (mean) of vaccinated animals (Complex III-III) are compared with control values (mean) of unvaccinated animals after different observation periods. Efficacy is 0%, 0%, 65.6% and 63.9% after 7, 16, 21 and 28 days respectively.

Figure 17. The dynamics of clinical symptoms of Trichophyton mentagrophytes infections in guinea pigs(Complex III-IV).
Severity score values (mean) of vaccinated animals (Complex III-IV) are compared with control values (mean) of unvaccinated animals after different observation periods. Efficacy is 10%, 2.3%, 44.5% and 74% after 7, 16, 21 and 28 days respectively.

Figure 18. The dynamics of clinical symptoms of Trichophyton mentagrophytes infections in guinea pigs (Complex III-V).
Severity score values (mean) of vaccinated animals (Complex III-V) are compared with control values (mean) of unvaccinated animals after different observation periods. Efficacy is 10%, 0%, 33.3% and 48.2% after 7, 16, 21 and 28 days respectively.

Figure 19. The dynamics of clinical symptoms of Trichophyton rubrum infections in rabbits (1st experiment).
Severity score values of vaccinated rabbits (Complex II-I) were 46.6%, 52.6%, 15.4% and 0% that of the values in the control group after 7, 15, 21 and 28 days respectively.

Figure 20. The dynamics of clinical symptoms of Trichophyton rubrum infections in rabbits (Complex II-I).
Severity score values (mean) of vaccinated rabbits (Complex II-I) are compared with control values (mean) of unvaccinated animals after different observation periods. Efficacy is 53.4%, 47.4%, 84.6%, and 100% after 7, 15, 21 and 28 days respectively.

**Examples:**

**Example 1**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456 were used to prepare 1.5 litres of vaccine. Trichophyton mentagrophytes DSM-7279 and Trichophyton rubrum DSM-9472 were cultivated separately on malt extract agar in 3 Roux flasks for each culture for 20 days at 28°C. Candida albicans DSM-9456 was cultivated in 2 Roux flasks on agar Sabouraud at 28°C for 3 days.
The fungal masses of the strains DSM-7279 and 9472 were lifted off and separately homogenised in 500 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The concentration of microconidia was adjusted to 60 million per ml for each homogenate. To yield 50 to 100% germ tubes each suspension of microconidia was fermented for 1-2 days at 28°C.
The blastospores of strain DSM-9456 were lifted off by washing with 500 ml of a physiological solution of sodium chloride. The concentration of blastospores in suspension was adjusted to 60 million per ml.
500 ml of each culture in suspension were combined and mixed in a single container. The homogenate was inactivated by adding thiomersal in a ratio of 1:20000 (w/v) directly to the cell suspension. For this purpose 75 mg of thiomersal were added to 1.5 litres of homogenate. The mixture was incubated at room temperature for 2 days.
The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection. The efficacy of the vaccine after Trichophyton rubrum challenge in guinea pigs and rabbits is shown in tables 9, 10, 11, 12, 13, 14, 15, 16 and figures 4, 5, 7, 8, 13, 14, 19, 20 (Complex II-I, III-I).

**Example 2**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456 were used to prepare 1.5 litres of vaccine. Trichophyton mentagrophytes DSM-7279 and Trichophyton rubrum DSM-9472 were cultivated separately on malt extract agar in 3 Roux flasks for each culture for 20 days at 28°C. The Candida albicans DSM-9456 was cultivated in 2 Roux flasks on agar Sabouraud at 28°C for 3 days.
The fungal masses of the strains DSM-7279 and 9472 were lifted off and separately homogenised in 500 ml of an aqueous solution 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1 % yeast extract. The concentration of microconidia was adjusted to 60 million per ml for each homogenate. To yield 50 to 100% germ tubes each suspension

of microconidia was fermented for 1-2 days at 28°C. Then the cell suspensions were washed with a physiological solution of sodium chloride 5 times by centrifugation (4000 rpm) at 10° C for 25 minutes for each centrifugation step.
The blastospores of strain DSM-9456 were lifted off by washing with 500 ml of a physiological solution of sodium chloride. The concentration of blastospores in suspension was adjusted to 60 million per ml.
Then 500 ml of each culture in suspension were combined and mixed in a single container. The homogenate was inactivated by adding thiomersal in a ratio of 1:20000 (w/v) directly to the cell suspension. For this purpose 75 mg of thiomersal were added to 1.5 litres of homogenate. The mixture was incubated at room temperature for 2 days.
The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection. The efficacy of the vaccine after Trichophyton rubrum challenge in guinea pigs is shown in tables 11, 12, 13, 14 and figures 7, 9, 13, 15 (Complex III-II).

**Example 3**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456 were used to prepare 1.5 litres of the vaccine. Trichophyton mentagrophytes DSM-7279 and Trichophyton rubrum DSM-9472 were cultivated separately on malt extract agar in 3 Roux flasks for each culture for 20 days at 28°C. The Candida albicans DSM-9456 was cultivated in 2 Roux flasks on agar Sabouraud at 28°C for 3 days.
The fungal masses of the strains DSM-7279 and 9472 were lifted off and separately homogenised in 500 ml of an aqueous solution of 0.5% soy peptone, 5% glucose and 0.1% yeast extract. The concentration of microconidia was adjusted to 65 million per ml for each homogenate. To yield 50 to 100% germ tubes each suspension of microconidia was fermented for 1-2 days at 28°C. The blastospores of strain DSM-9456 were lifted off by washing with 500 ml of physiological solution of sodium chloride. The concentration of blastospores in suspension was adjusted to 60 million per ml.
500 ml of each culture in suspension were combined and mixed in a single container. The homogenate was inactivated by adding thiomersal in a ratio 1:20000 (w/v) directly to the cell suspension. For this purpose 75 mg of thiomersal were added to 1.5 litres of homogenate. The mixture was incubated at room temperature for 2 days.
The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection. The efficacy of the vaccine after Trichophyton rubrum challenge in guinea pigs is shown in tables 11, 12, 13, 14 and figures 7, 10, 13, 16 (Complex III-III)

**Example 4**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456 were used to prepare 1.5 litres of the vaccine. Trichophyton mentagrophytes DSM-7279 and Trichophyton rubrum DSM-9472 were cultivated separately on malt extract agar in 3 Roux flasks for each culture for 20 days at 28°C. The Candida albicans DSM-9456 was cultivated in 2 Roux flasks on agar Sabouraud at 28°C for 3 days.
The fungal masses of the strains DSM-7279 and 9472 were lifted off and separately homogenised in 500 ml of an aqueous solution of 0.5% soya peptone, 5% glucose and 0.1 % yeast extract. The concentration of microconidia was adjusted to 55 million per ml for each homogenate. To yield 50 to 100% germ tubes each suspension of microconidia was fermented for 1-2 days at 28°C. The cell suspensions were washed with a physiological solution of sodium chloride 5 times by centrifugation (4000 rpm) at 10°C for 25 minutes for each centrifugation step.
The blastospores of strain DSM-9456 were lifted off by washing with 500 ml of a physiological solution of sodium chloride. The concentration of blastospores in suspension was adjusted to 60 million per ml.
500 ml of each culture in suspension were combined and mixed in a single container. The homogenate was inactivated by adding thiomersal in a ratio of 1:20000 (w/v) directly to the cell suspension. For this purpose 75 mg of thiomersal were added to 1.5 litres of homogenate. The mixture was incubated at room temperature for 2 days.
The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection. The efficacy of the vaccine after Trichophyton rubrum challenge in guinea pigs is shown in tables 11, 12, 13, 14 and figures 7, 11, 13, 17 (Complex III-IV).

**Example 5**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9469, Trichophyton rubrum DSM-9470, Trichophyton rubrum DSM-9471, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456, Candida albicans DSM-9457, Candida albicans DSM-9456, Candida albicans DSM-9459 were used to prepare 1.5 litres of the vaccine. Cultures of strains DSM-7279, 9469, 9470, 9471,9472 were cultivated separately on malt

extract agar in 3 Roux flasks for each culture for 20 days at 28°C. Cultures of Candida albicans strains DSM-9456, 9457, 9458, 9459 were cultivated in 1 Roux flask for each culture on agar Sabouraud at 28°C for 3 days.
The fungal masses of strains DSM-9469, 9470, 9471 and 9472 were lifted off and separately homogenised in 100 ml of a solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. Then the fungal mass of strain DSM-7279 was homogenised in 500 ml of a solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The concentration of microconidia was adjusted to 60 million per ml for each homogenate. To yield 50 to 100% germ tubes in suspensions of microconidia each dermatophyte culture was fermented for 1 day at 28°C. After cultivation 150 ml of each suspension of Trichophyton rubrum DSM-9469, 9470, 9471, 9472 were mixed in a single container.
The blastospores of strains DSM-9456, 9457, 9458, 9459 were lifted off by washing with 200 ml of a physiological solution of sodium chloride. The concentration of blastospores in each suspension was adjusted to 60 million per ml. 150 ml of each suspension were mixed in a single container.
500 ml of the Trichophyton mentagrophytes DSM-7279 suspension were mixed with 500 ml of the Trichophyton rubrum DSM-9469, 9470, 9471, 9472 mixture suspension and with 500 ml of the Candida albicans DSM-9456, 9457, 9458, 9459 mixture suspension in a single container. The homogenate was inactivated by adding thiomersal in a ratio of 1:12500 (w/v) directly to the cell suspension. For this purpose 80 mg of thiomersal were added to 1 litre of homogenate. The mixture was incubated at room temperature for 1 day.
The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection.

**Example 6**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9469, Trichophyton rubrum DSM-9471, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456, Candida albicans DSM-9457 were used to prepare 1.5 litres of the vaccine. Cultures of strains DSM-7279, 9469, 9471, 9472 were cultivated separately on malt extract agar in 4 Roux flasks for each culture for 20 days at 28°C. Cultures of Candida albicans strains DSM-9456, 9457 were cultivated in 1 Roux flask for each culture on agar Sabouraud at 28°C for 3 days.
The fungal masses of strains DSM-9469, 9471 and 9472 were lifted off and each culture separately homogenised in 200 ml of a solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1 % yeast extract. The fungal mass of strain DSM-7279 was homogenised in 500 ml of a solution a 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The concentration of microconidia was adjusted to 60 million per ml of the homogenate for each culture. To yield 50 to 100% germ tubes in suspensions of microconidia each of dermatophyte strains was fermented for 1 day at 28°C. After cultivation 200 ml of Trichophyton rubrum DSM-9469, 9471, 9472 suspensions were mixed in a single container.
The blastospores of strain DSM-9456, 9457 were lifted off by washing with 250 ml of a physiological solution of sodium chloride. The concentration of blastospores in suspension was adjusted to 60 million per ml.
250 ml of the of each culture suspension were combined and mixed in a single container. 500 ml of the Trichophyton mentagrophytes DSM-7279 suspension were mixed with 500 ml of the Trichophyton rubrum DSM-9469, 9471, 9472 mixture suspension and with 500 ml of the suspensions of cultures DSM-9456, 9457 in a single container. The homogenates were inactivated by adding thiomersal in a ratio of 1:25000 (w/v) directly to the cell suspension. For this purpose 60 mg of thiomersal were added to 1.5 litres of homogenate. The mixture was incubated at room temperature for 2 days.
The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection.

**Example 7**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456, Candida albicans DSM-9457 were used to prepare 1.5 litres of the vaccine. Cultures of strains DSM-7279, 9472 were cultivated separately on malt extract agar in 8 Roux flasks for each culture for 20 days at 28°C. Cultures of Candida albicans strains DSM-9456, 9457 were cultivated in 1 Roux flask for each culture on agar Sabouraud at 28°C for 3 days.
Fungal masses of strain DSM-9472 were lifted off and homogenised in 500 ml of a solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The fungal mass of strain DSM-7279 was homogenised in 500 ml of a solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The concentration of microconidia was then adjusted to 50 million per ml of homogenate for each culture. To yield 50 to 100% germ tubes in suspensions of microconidia each strain of dermatophytes was fermented for 2 days at 28°C.

The blastospores of strain DSM-9456, 9457 were lifted off by washing with 250 ml of a physiological solution of sodium chloride. The concentration of blastospores in suspension was adjusted to 60 million per ml. 250 ml of each culture suspension were combined and mixed in a single container.
500 ml of the Trichophyton mentagrophytes DSM-7279 suspension were mixed with 500 ml of the Trichophyton rubrum DSM-9472 suspension and with 500 ml of the suspensions of cultures DSM-9456, 9457 in a single container. The homogenates were inactivated by adding thiomersal in a ratio of 1:12500 (w/v) directly to the cell suspension. For this purpose 120 mg of thiomersal were added to 1.5 litres of homogenate. The mixture was incubated at room temperature for 2 days.
The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection.

**Example 8**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456 were used to prepare 1.5 litres of the vaccine. Cultures of strains DSM-7279, 9472 were cultivated separately on malt extract agar in 6 Roux flasks for each culture for 20 days at 28°C. Cultures of Candida albicans strain DSM-9456 were cultivated in 2 Roux flasks on agar Sabouraud at 28°C for 3 days.
Fungal masses of strains DSM-7279 and 9472 were lifted off and separately homogenised in 500 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The concentration of microconidia was adjusted to 60 million per ml for each homogenate. To yield 50 to 100% germ tubes both suspensions of microconidia were fermented for 2 days at 28°C. 500 ml of the Trichophyton mentagrophytes DSM-7279 suspension were mixed with 500 ml of the Trichophyton rubrum DSM-9472 suspension in a single container. The homogenates were inactivated by adding thiomersal in a ratio of 1:25000 (w/v) directly to the cell suspension. For this purpose 40 mg of thiomersal were added to 1 litre of homogenate. The mixture was incubated at room temperature for 2 days.
The culture of strain DSM-9456 was harvested and homogenised in 5000 ml medium RPMI No. 1640 with L-glutamine (Serva). The concentration of the blastospores was adjusted to 20 million per ml. 5000 ml of this cell suspension was incubated in cell culture flasks containing medium No. 1640 in a $CO_2$ atmosphere of 5% at 36-38°C. After 4 hours incubation period 50 % to 100% of the blastospores commonly displayed germ tubes and a swollen condition.
The blastospores were harvested and washed 3 times by centrifugation (4000 rpm) at 4-10°C for 25 minutes for each centrifugation step. The concentration of the cells was adjusted to 60 million per ml. The homogenates were inactivated by adding thiomersal in a ratio of 1:20000 (w/v) directly to the cell suspension.
For this purpose 80 mg of thiomersal were added to 1 litre of homogenate. The mixture was incubated at room temperature for 2 days.
750 ml of the suspension of culture of DSM-9456 were combined and mixed with 750 ml of the mixture of DSM-7279 and 9472 culture suspension. The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection. The efficacy of the vaccine in mice after Candida albicans challenge is shown in tables 1, 2, 5 and after Trichophyton rubrum challenge in guinea pigs in tables 7, 8 and figures 1, 2 (Complex I-I, 1-I, 3-I)

**Example 9**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9469, Trichophyton rubrum DSM-9470, Trichophyton rubrum DSM- 9471, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456, Candida albicans DSM-9457, Candida albicans DSM-9458, Candida albicans DSM-9459 were used to prepare 1.5 litres of the vaccine. Cultures of strains DSM-7279, 9469, 9470, 9471, 9472 were cultivated separately on malt extract agar in 3 Roux flasks for each culture for 20 days at 28°C. Cultures of Candida albicans strains DSM-9456, 9457, 9458, 9459 were cultivated in 1 Roux flask for each culture on agar Sabouraud at 28°C for 3 days.
Fungal masses of strains DSM-9469, 9470, 9471 and 9472 were lifted off and separately homogenised in 100 ml of a solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1 % yeast extract. The fungal mass of strain DSM- 7279 was homogenised in 500 ml of a solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The concentration of microconidia was adjusted to 60 million per ml for each homogenate. To yield 50 to 100% germ tubes in suspensions of microconidia each dermatophyte culture was fermented for 1 day at 28°C.
After cultivation 150 ml of each suspension of Trichophyton rubrum DSM 9469, 9470, 9471, 9472 were mixed in a single container. The homogenates were inactivated by adding thiomersal in a ratio of 1:16000 (w/v) directly to the cell suspensions. For this purpose 62.5 mg of thiomersal were added to 1 litre of homogenate. The mixture was incubated at room temperature for 2 days.

The culture of strain DSM-9456, 9457, 9458, 9459 were harvested and homogenised in medium No. 1640 (Serva). The concentration of the blastospores was adjusted to 20 million per ml. 1500 ml of cell suspensions of each culture were incubated in cell culture flasks containing medium No. 1640 in a $CO_2$ atmosphere of 6% at 36-38° C. After 3 hours incubation period 50 % to 100% of the blastospores commonly displayed germ tubes and a swollen condition. The blastospores were harvested and washed 3 times by centrifugation (4000 rpm) at 4-10°C for 25 minutes for each centrifugation step. The concentration of the cells was adjusted to 60 million per ml. The homogenate was inactivated by adding thiomersal in a ratio of 1:25000 (w/v) directly to the cell suspension. For this purpose 40 mg of thiomersal were added to 1 litre of homogenate. The mixture was incubated at room temperature for 1 day. 150 ml of each suspension of the cultures of DSM-9456, 9457, 9458, 9459 were combined and mixed in a single container.
500 ml of the mixture suspensions of cultures DSM-9469, 9470, 9471, 9472 were mixed with 500 ml suspension culture DSM-7279 and 500 ml mixture suspensions of cultures DSM-9456, 9457, 9458, 9459.
The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection.

**Example 10**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9469, Trichophyton rubrum DSM-9471, Trichophyton rubrum DSM- 9472 and Candida albicans DSM-9456, Candida albicans DSM-9457 were used to prepare 1.5 litres of the vaccine. Cultures of strains DSM-7279, 9469, 9471, 9472 were cultivated separately on malt extract agar in 4 Roux flasks for each culture for 20 days at 28°C. Cultures of Candida albicans strains DSM-9456, 9457 were cultivated in 1 Roux flask for each culture on agar Sabouraud at 28°C for 3 days.
Fungal masses of strains DSM-9469, 9471 and 9472 were lifted off, combined and homogenised in 100 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1 % yeast extract. The fungal masses of strain DSM-7279 and the mixture of strains DSM-9469, 9471 and 9472 were lifted off and each homogenised in 500 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The concentration of microconidia was adjusted to 60 million per ml of the homogenate. To yield 50 to 100% germ tubes both suspensions of microconidia were fermented for 1-2 days at 28°C. 500 ml of the Trichophyton mentagrophytes DSM-7279 suspension were mixed with 500 ml of the mixture of Trichophyton rubrum DSM-9469, 9471, 9472 suspension in a single container. The homogenate was inactivated by adding thiomersal in a ratio of 1:20000 (w/v) directly to the cell suspension. For this purpose 50 mg of thiomersal were added to 1 litre of homogenate. The mixture was incubated at room temperature for 2 days.
The cultures of strains DSM-9456, 9457 were harvested and homogenised in medium No. 1640 (Serva). The concentration of the blastospores was adjusted to 20 million per ml. 1500 ml of cell suspensions of each cultures were incubated in cell culture flasks containing medium No. 1640 in a $CO_2$ atmosphere of 5% at 36-38°C. After 4 hours incubation period 50 to 100% of the blastospores commonly displayed germ tubes and a swollen condition. The blastospores were harvested and washed 3 times by centrifugation (5000 rpm) at 4-10°C for 20 minutes for each centrifugation step. The concentration of the cells was adjusted to 50 million per ml. The homogenate was inactivated by adding thiomersal in a ratio of 1:20000 (w/v) directly to the cell suspension. For this purpose 50 mg of thiomersal were added to 1 litre of homogenate. The mixture was incubated at room temperature for 1 day. 150 ml of each suspension of cultures DSM-9456, 9457 were combined and mixed in a single container.
500 ml of the mixture suspensions of culture DSM-9469, 9471, 9472 were mixed with 500 ml suspension culture DSM-7279 and with 500 ml mixture suspensions of cultures DSM-9456, 9457.
The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection.

**Example 11**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456, Candida albicans DSM-9457, Candida albicans DSM-9459 were used to prepare 1.5 litres of the vaccine. Cultures of strains DSM-7279, 9472 were cultivated separately on malt extract agar in 6 Roux flasks for each culture for 20 days at 28°C. Cultures of Candida albicans strains DSM-9456, 9457, 9459 were cultivated in 1 Roux flask each on agar Sabouraud at 28°C for 3 days.
The fungal masses of strains DSM-7279 and 9472 were lifted off and separately homogenised in 500 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The concentration of microconidia was adjusted to 60 million per ml of the homogenate. To yield 50 to 100% germ tubes both suspensions of microconidia were fermented for 1 day at 28°C. 500 ml of the Trichophyton mentagrophytes DSM-7279 suspension

were mixed with 500 ml of the Trichophyton rubrum DSM-9472 suspension in a single container. The homogenate was inactivated by adding thiomersal in a ratio of 1:20000 (w/v) directly to the cell suspension. For this purpose 50 mg of thiomersal were added to 1 litre of homogenate. The mixture was incubated at room temperature for 2 days.

The cultures of Candida albicans strains DSM-9456, 9457, 9459 were harvested and separately homogenised in medium No. 1640 (Serva). The concentration of the blastospores was adjusted to 10 million per ml. 2000 ml of each cell suspension was incubated separately in cell culture flasks or in Petri dishes containing medium No. 1640 in a $CO_2$ atmosphere of 6% at 38°C. After 3 hours incubation period 50-100% of the blastospores commonly displayed germ tubes or a swollen condition. The blastospores were harvested and washed 3 times by centrifugation (5000 rpm) at 4-10°C for 25 minutes for each centrifugation step. The concentration of the cells was adjusted to 20 million per ml. The cell suspensions of each strain were mixed using equal volumes. The mixed suspension was inactivated with thiomersal in a ratio of 1:25000 (w/v).

500 ml of this suspension was mixed with 1000 ml suspension of microconidiae. The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. Vaccine prepared in this manner was used to immunise animals by intramuscular injection.

**Example 12**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456 were used to prepare 1.5 litres of the vaccine. Cultures of strains DSM-7279, 9472 were cultivated separately on malt extract agar in 6 Roux flasks for each culture for 20 days at 28°C. Cultures of Candida albicans strain DSM-9456 were cultivated in 2 Roux flasks on agar Sabouraud at 28°C for 3 days.

The fungal masses of strains DSM-7279 and 9472 were lifted off and separately homogenised in 500 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The concentration of microconidia was adjusted to 60 million per ml of the homogenate. To yield 50 to 100% germ tubes both suspensions of microconidia were fermented for 2 days at 28°C. 500 ml of the Trichophyton mentagrophytes DSM-7279 suspension were mixed with 500 ml of the Trichophyton rubrum DSM-9472 suspension in a single container.

The blastospores of strain DSM-9456 were lifted off by washing with 500 ml of destilled water. The concentration of blastospores in suspension was adjusted to 56 million per ml.

500 ml of this suspension were mixed with the suspension of microconidiae.

The homogenate was inactivated by adding thiomersal in a ratio of 1:20000 (w/v) directly to the cell suspension. For this purpose 50 mg thiomersal were added to 1 litre of homogenate. This mixture was incubated at room temperature for 2 days.

After the inactivating process the cell suspension was treated with $H_2O_2$. A substance containing $H_2O_2$, for example Urea-hydrogen peroxide, was added to a cell suspension to yield a final concentration of 3% of $H_2O_2$. This cell suspension was stirred for 24 hours. Treated cells were washed 5 times for 30 minutes with destilled water by centrifugation (4000 rpm). The final concentration of cells was adjusted to 60 million per ml.

The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. Vaccine prepared in this manner was used to immunise animals by intramuscular injection. The efficacy of the vaccine after Candida albicans challenge in mice is shown in table 6, and after Trichophyton rubrum challenge in guinea pigs in tables 11, 12, 13,14 and figures 7, 12, 13, 18 (Complex 4-I; III-V).

**Example 13**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9469, Trichophyton rubrum DSM-9470, Trichophyton rubrum DSM-9471, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456, Candida albicans DSM-9457, Candida albicans DSM-9458, Candida albicans DSM-9459 were used to prepare 1.5 litres of the vaccine. Cultures of strains DSM-7279, 9469, 9470, 9471, 9472 were cultivated separately on malt extract agar in 3 Roux flasks for each culture for 20 days at 28°C. Cultures of Candida albicans strains DSM-9456, 9457, 9458, 9459 were cultivated in 1 Roux flask for each culture on agar Sabouraud at 28°C for 3 days.

The fungal masses of strains DSM-9469, 9470, 9471 and 9472 were lifted off, combined and homogenised in 100 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The fungal masses of strain DSM-7279 and the mixture of strains DSM-9469, 9470, 9471 and 9472 were lifted off and separately homogenised in 500 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The concentration of microconidia was adjusted to 60 million per ml of the homogenate. To yield 50 to 100% germ tubes both suspensions of microconidia were fermented for 1-2 days at 28°C. 500 ml of the Trichophyton mentagrophytes DSM-7279 suspension were mixed with 500 ml of the mixture of Trichophyton rubrum DSM-9469, 9470, 9471, 9472 suspension in a single container.

The blastospores of strain DSM-9456, 9457, 9458, 9459 were lifted off by washing with 200 ml of destilled water. The

concentration of blastospores in suspension was adjusted to 60 million per ml. 150 ml of each suspension were mixed. 500 ml of the resulting suspension were mixed with the suspension of microconidiae. The homogenate was inactivated by adding thiomersal directly to the cell suspension in a ratio of 1:20000 (w/v). For this purpose 50 mg thiomersal were added to 1 litre of homogenate. This mixture was incubated at room temperature for 2 days. Following inactivation the cell suspension was treated with sodium permanganate in a concentration of 1:10000 (w/v) for 16 hours while stirring. Treated cells were washed 5 times with destilled water by centrifugation (4000 rpm) for 25 minutes for each centrifugation step. The final concentration of cells was adjusted to 40 million per ml.
The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection.

**Example 14**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9469, Trichophyton rubrum DSM-9471, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456, Candida albicans DSM-9457 were used to prepare 1.5 litres of the vaccine. Cultures of strains DSM-7279, 9469, 9471, 9472 were cultivated separately on malt extract agar in 4 Roux flasks for each culture for 20 days at 28°C. Cultures of Candida albicans strains DSM-9456, 9457 were cultivated in 1 Roux flask for each culture on agar Sabouraud at 28°C for 3 days.
The fungal masses of strains DSM-9469, 9471 and 9472 were lifted off, combined and homogenised in 100 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The fungal masses of strain DSM-7279 and the mixture of strains DSM-9469, 9471 and 9472 were lifted off and separately homogenised in 500 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1 % yeast extract. The concentration of microconidia was adjusted to 60 million per ml of the homogenate. To yield 50 to 100% germ tubes both suspensions of microconidia were fermented for 1-2 days at 28°C. 500 ml of the Trichophyton mentagrophytes DSM-7279 suspension were mixed with 500 ml of the mixture of Trichophyton rubrum DSM-9469, 9471, 9472 suspension in a single container.
The blastospores of strain DSM-9456 and 9457 were lifted off by washing with 200 ml of a physiological sodium chloride solution. The concentration of blastospores in suspension was adjusted to 60 million per ml. 250 ml of each suspension were mixed.
500 ml of the resulting suspension was mixed with a suspension of microconidiae. The homogenate was inactivated by adding thiomersal directly to the cell suspension in a ratio of 1:20000 (w/v). For this purpose 50 mg thiomersal were added to 1 litre of homogenate. This mixture was incubated at room temperature for 2 days.
After the inactivating process the cell suspension was treated with $H_2O_2$. hydrogen peroxide tablets (Wasserstoff-Peroxid Tabletten WDT) were added to cell suspensions to yield a final concentration of 1% of $H_2O_2$. The cell suspension was stirred for 24 hours. Treated cells were washed 5 times for 30 minutes with destilled water by centrifugation (4000 rpm). The final concentration of the cells was adjusted to 50 million per ml.
The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection.

**Example 15**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456, Candida albicans DSM-9457, Candida albicans DSM-9459 were used to prepare 1.5 litres of the vaccine. Cultures of strains DSM-7279, 9472 were cultivated separately on malt extract agar in 6 Roux flasks for each culture for 20 days at 28°C. Cultures of Candida albicans strains DSM-9456, 9457, 9459 were cultivated in 1 Roux flask for each culture on agar Sabouraud at 28°C for 3 days.
The fungal masses of strains DSM-7279 and 9472 were lifted off and separately homogenised in 500 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The concentration of microconidia was adjusted to 60 million per ml of the homogenate. To yield 50 to 100% germ tubes both suspensions of microconidia were fermented for 1-2 days at 28°C. 500 ml of the Trichophyton mentagrophytes DSM-7279 suspension were mixed with 500 ml of the Trichophyton rubrum DSM 9472 suspension in a single container.
The blastospores of strain DSM-9456, 9457, 9459 were lifted off by washing with 200 ml of a physiological sodium chloride solution. The concentration of blastospores in suspension was adjusted to 60 million per ml. 250 ml of each suspension were mixed.
500 ml of the resulting suspension were mixed with the suspension of microconidiae. The homogenate was inactivated by adding thiomersal directly to the cell suspension in a ratio of 1:20000 (w/v). For this purpose 50 mg thiomersal were added to 1 litre of homogenate. This mixture was incubated at room temperature for 2 days.

Following the inactivation the cell suspension was treated with sodium permanganate in a concentration of 1:20000 (w/v) for 36 hours while stirring. Treated cells were washed 5 times with destilled water by centrifugation (4000 rpm) for 25 minutes for each centrifugation step.
The final concentration of the cells was adjusted to 60 million per ml. The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection.

**Example 16**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456 were used to prepare 1.5 litres of the vaccine. Cultures of strains DSM-7279, 9472 were cultivated separately on malt extract agar in 6 Roux flasks for each culture for 20 days at 28°C. Cultures of Candida albicans strain DSM-9456 were cultivated in 2 Roux flasks on agar Sabouraud at 28°C for 3 days.
The fungal masses of strains DSM-7279 and 9472 were lifted off and separately homogenised in 200 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The concentration of microconidia was adjusted to 60 million per ml of the homogenate. To yield 50 to 100% germ tubes both suspensions of microconidia were fermented for 1-2 days at 28°C. 500 ml of the Trichophyton mentagrophytes DSM-7279 suspension were mixed with 500 ml of the Trichophyton rubrum DSM-9472 suspension in a single container. The homogenate was inactivated by adding thiomersal in a ratio of 1:20000 (w/v) directly to the cell suspension. For this purpose 50 mg of thiomersal were added to 1 litre of homogenate. The mixture was incubated at room temperature for 2 days.
Following inactivation the cell suspension was treated with $H_2O_2$. A substance containing $H_2O_2$, for example Urea-hydrogen peroxide, was added to the cell suspension to yield a final concentration of 3% of $H_2O_2$. This cell suspension was stirred for 24 hours. Treated cells were washed 5 times for 30 minutes with destilled water by centrifugation (4000 rpm). The final concentration of cells was adjusted to 40 million per ml.
The culture of strain DSM-9456 was harvested and homogenised in medium No. 1640 (Serva). The concentration of the blastospores was adjusted to 20 million per ml. 2000 ml of this cell suspension were incubated in cell culture flasks of medium No. 1640 in a $CO_2$ atmosphere of 5% at 36-38°C. After 3 hours incubation period 50 % to 100% of the blastospores commonly displayed germ tubes or a swollen condition. The blastospores were harvested and washed for 3 times by centrifugation (4000 rpm) at 4-10°C for 25 minutes for each centrifugation step. The concentration of the cells was adjusted to 40 million per ml. The suspension was inactivated using thiomersal in a ratio of 1:25000 (w/v).
Following the inactivation the cell suspension was treated with $H_2O_2$. A substance containing $H_2O_2$, for example Urea-hydrogen peroxide (Wasserstoff-Peroxid Harnstoff zur Synthese $CN_2H_4O$ $H_2O_2$), was added to cell suspensions to yield a final concentration of 3% of $H_2O_2$. The cell suspension was stirred for 24 hours. Treated cells were washed 5 times for 30 minutes with destilled water by centrifugation (4000 rpm). The final concentration of cells was adjusted to 120 million per ml. 500 ml of this suspension were mixed with 1000 ml suspension of microconidiae.
The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection. The efficacy of the vaccine after Candida albicans challenge in mice is shown in tables 1, 2, 3, 4, and after Trichophyton rubrum challenge in guinea pigs in tables 7, 8 and figures 1, 3 (Complex 1-II; 2-I; I-II).

**Example 17**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9469, Trichophyton rubrum DSM-9470, Trichophyton rubrum DSM-9471, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456, Candida albicans DSM-9457, Candida albicans DSM-9458, Candida albicans DSM-9459 were used to prepare 1.5 litres of the vaccine. Cultures of strains DSM-7279, 9469, 9470, 9471, 9472 were cultivated separately on malt extract agar in 3 Roux flasks for each culture for 20 days at 28°C. Cultures of Candida albicans strains DSM-9456, 9457, 9458, 9459 were cultivated in 1 Roux flask for each culture on agar Sabouraud at 28°C for 3 days.
The fungal masses of strains DSM-9469, 9470, 9471 and 9472 were lifted off, combined and homogenised in 100 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The fungal masses of strain DSM-7279 and the mixture of strains DSM-9469, 9470, 9471 and 9472 were lifted off and separately homogenised in 500 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The concentration of microconidia was adjusted to 60 million per ml of the homogenate. To yield 50 to 100% germ tubes both suspensions of microconidia were fermented for 1-2 days at 28°C. 500 ml of the Trichophyton mentagrophytes DSM-7279 suspension were mixed with 500 ml of the mixture of Trichophyton rubrum DSM-9469, 9470, 9471, 9472 suspension in a single container. The homogenate was inactivated by adding thiomersal in a ratio of 1:20000 (w/v) directly to the cell suspension. For this purpose 50 mg of thiomersal were added to 1 litre of homogenate. The mixture was incubated at room temperature for 2 days.

Following the inactivation the cell suspension was treated with sodium permanganate in a concentration of 1:30000 (w/v) for 24 hours while stirring. Treated cells were washed 5 times with destilled water by centrifugation (4000 rpm) for 25 minutes for each centrifugation step. The final concentration of the cells was adjusted to 60 million per ml.

The cultures of strains DSM-9456, 9457, 9458, 9459 were harvested and separately homogenised in medium No. 1640. The concentration of the blastospores was adjusted to 20 million per ml. 130 ml of the cell suspensions were incubated separately in cell culture flasks containing medium No. 1640 in a $CO_2$ atmosphere of 5% at 36-38°C. After 3 hours incubation period 50 % to 100% of the blastospores commonly displayed germ tubes or a swollen condition. The blastospores were harvested and washed for 2-3 times by centrifugation (4000 rpm) at 4-10°C for 25 minutes for each centrifugation step. The concentration of the cells was adjusted to 40 million per ml. The suspension was inactivated using thiomersal in a ratio of 1:25000 (w/v). Following the inactivation the cell suspension was treated with $H_2O_2$. Hydrogen peroxide tablets (Wasserstoff-Peroxid Tabletten WDT) were added to cell suspensions to yield a final concentration of 3% of $H_2O_2$. The cell suspension was stirred for 24 hours. Treated cells were washed 5 times for 30 minutes with destilled water by centrifugation (4000 rpm). The final concentration of the cells was adjusted to 60 million per ml. 500 ml of this suspension were mixed with 1000 ml suspension of microconidiae. The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection.

**Example 18**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9469, Trichophyton rubrum DSM-9471, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456, Candida albicans DSM-9457 were used to prepare 1.5 litres of the vaccine. Cultures of strains DSM-7279, 9469, 9471, 9472 were cultivated separately on malt extract agar in 4 Roux flasks for each culture for 20 days at 28°C. Cultures of Candida albicans strains DSM-9456, 9457 were cultivated in 1 Roux flask for each culture on agar Sabouraud at 28°C for 3 days.

The fungal masses of strains DSM-9469, 9471 and 9472 were lifted off, combined and homogenised in 100 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1 % yeast extract. The fungal mass of strain DSM-7279 and the mixture of strains DSM-9469, 9471 and 9472 were lifted off, separately homogenised in 500 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The concentration of microconidia was adjusted to 60 million per ml of the homogenate. To yield 50 to 100% germ tubes both suspensions of microconidia were fermented for 1-2 days at 28°C. 500 ml of the Trichophyton mentagrophytes DSM-7279 suspension were mixed with 500 ml of the mixture of Trichophyton rubrum DSM-9469, 9471, 9472 suspension in a single container. The homogenate was inactivated by adding thiomersal in a ratio of 1:20000 (w/v) directly to the cell suspension. For this purpose 50 mg of thiomersal were added to 1 litre of homogenate. The mixture was incubated at room temperature for 2 days.

Following the inactivation the cell suspension was treated with $H_2O_2$. Hydrogen peroxide tablets (Wasserstoff-Peroxid Tabletten WDT) were added to the cell suspension to yield a final concentration of 2% of $H_2O_2$. The cell suspension was stirred for 36 hours. Treated cells were washed 5 times for 25 minutes with destilled water by centrifugation (4000 rpm). The final concentration of cells was adjusted to 60 million per ml.

The cultures of strains DSM-9456, 9457 were harvested and separately homogenised in medium No. 1640 (Serva). The concentration of the blastospores was adjusted to 10-20 million per ml. 130 ml of the cell suspensions were incubated separately in cell culture flasks containing medium No. 1640 in a $CO_2$ atmosphere of 6 % at 36-38°C. After 3 hours incubation period 50 % to 100% of the blastospores commonly displayed germ tubes or a swollen condition. The blastospores were harvested and washed for 3 times by centrifugation (4000 rpm) at 4-10°C for 25 minutes for each centrifugation step. The concentration of the cells was adjusted to 40 million per ml. The suspension was inactivated using thiomersal in a ratio of 1:25000 (w/v). Following the inactivation the cell suspension was treated with $H_2O_2$. Hydrogen peroxide tablets (Wasserstoff-Peroxid Tabletten WDT) were added to the cell suspension to yield a final concentration of 3% of $H_2O_2$. This cell suspension was stirred for 24 hours. Treated cells were washed 5 times for 30 minutes with destilled water by centrifugation (4000 rpm). The final concentration of cells was adjusted to 60 million per ml. 500 ml of this suspension were mixed with 1000 ml suspension of microconidiae. The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection.

**Example 19**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456, Candida albicans DSM-9457, Candida albicans DSM-9459 were used to prepare 1.5 litres of the vaccine. Cultures of strains DSM-7279, 9472 were cultivated separately on malt extract agar in 6 Roux flasks for each culture for 20 days at 28°C. Cultures of Candida albicans strains DSM-9456, 9457, 9459 were cultivated in 1 Roux flask

for each culture on agar Sabouraud at 28°C for 3 days.
The fungal masses of strains DSM-7279 and 9472 were lifted off and separately homogenised in 500 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The concentration of microconidia was adjusted to 60 million per ml of the homogenate. To yield 50 to 100% germ tubes both suspensions of microconidia were fermented for 1-2 days at 28°C. 500 ml of the Trichophyton mentagrophytes DSM-7279 suspension were mixed with 500 ml of the Trichophyton rubrum DSM-9472 suspension in a single container. The homogenate was inactivated by adding thiomersal in a ratio of 1:20000 (w/v) directly to the cell suspension. For this purpose 50 mg of thiomersal were added to 1 litre of homogenate. The mixture was incubated at room temperature for 2 days.
Following the inactivation the cell suspension was treated with sodium permanganate in a concentration of 1:30000 (w/v) for 24 hours while stirring. Treated cells were washed 5 times with destilled water by centrifugation (4000 rpm) for 25 minutes for each centrifugation step. The final concentration of cells was adjusted to 40 million per ml.
The cultures of Candida albicans strains DSM-9456, 9457, 9459 were harvested and separately homogenised in medium No. 1640 (Serva). The concentration of the blastospores was adjusted to 20 million per ml. 130 ml of each cell suspension were incubated separately in cell culture flasks containing medium No. 1640 in a $CO_2$ atmosphere of 6% at 36-38°C. After 4 hours incubation period 50 % to 100% of the blastospores commonly displayed germ tubes or a swollen condition. The blastospores were harvested and washed for 3 times by centrifugation (4000 rpm) at 4-10° C for 30 minutes for each centrifugation step. The concentration of the cells was adjusted to 60 million per ml. The cell suspensions of each strain were mixed using equal volumes. The mixed suspension was inactivated using thiomersal in a ratio of 1:11000 (w/v).
Following the inactivation the cell suspension was treated with sodium permanganate in a concentration of 1:20000 (w/v) for 24 hours while stirring. Treated cells were washed 5 times with destilled water by centrifugation (4000 rpm) for 25 minutes for each centrifugation step. The final concentration of the cells was adjusted to 60 million per ml.
500 ml of this suspension were mixed with 1000 ml suspension of microconidiae. The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection.

**Example 20**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456 were used to prepare 1.5 litres of the vaccine. Trichophyton mentagrophytes DSM-7279 and Trichophyton rubrum DSM-9472 were cultivated separately on malt extract agar in 6 Roux flasks for each culture for 20 days at 28°C. The Candida albicans DSM-9456 was cultivated in 2 Roux flasks on agar Sabouraud at 28°C for 3 days.
The fungal masses of the strains DSM-7279 and 9472 were lifted off and separately homogenised in 500 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1 % yeast extract. The concentration of microconidia was adjusted to 60 million per ml of the homogenate. 500 ml of each suspension were mixed in a single container.
The blastospores of strain DSM-9456 were lifted off by washing with 500 ml of destilled water. The concentration of blastospores in suspension was adjusted to 60 million per ml.
500 ml of this suspension were mixed with the suspension of microconidiae. The homogenate was inactivated by adding thiomersal in a ratio of 1:20000 (w/v) directly to the cell suspension. For this purpose 50 mg of thiomersal were added to 1 litre of homogenate. The mixture was incubated at room temperature for 2 days.
The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection. The efficacy of the vaccine after Trichophyton rubrum challenge in guinea pigs is shown in tables 9, 10 and figures 4 and 6 (Complex II-II).

**Example 21**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9469, Trichophyton rubrum DSM-9470, Trichophyton rubrum DSM- 9471, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456, Candida albicans DSM-9457, Candida albicans DSM-9458, Candida albicans DSM-9459 were used to prepare 1.5 litres of the vaccine. Cultures of strains DSM-7279, 9469, 9470, 9471, 9472 were cultivated separately on malt extract agar in 3 Roux flasks for each culture for 20 days at 28°C. Cultures of Candida albicans strains DSM-9456, 9457, 9458, 9459 were cultivated in 1 Roux flask for each culture on agar Sabouraud at 28°C for 3 days.
The fungal masses of strains DSM-9469, 9470, 9471 and 9472 were lifted off, combined and homogenised in 100 ml of an aqueous solution of 3% fermented hydrolyzed muscle protein, 5% glucose and 1% yeast extract. The fungal masses of strain DSM-7279 and the mixture of strains DSM-9469, 9470, 9471 and 9472 were lifted off and separately homogenised in 500 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1%

yeast extract. The concentration of microconidia was adjusted to 60 million per ml of the homogenate. 500 ml of the Trichophyton mentagrophytes DSM-7279 suspension were mixed with 500 ml of the Trichophyton rubrum DSM-9469, 9470, 9471, 9472 mixture suspension in a single container.
The blastospores of strain DSM-9456, 9457, 9458, 9459 were lifted off by washing with 200 ml of destilled water. The concentration of blastospores in suspension was adjusted to 60 million per ml. 150 ml of each suspension were mixed. 500 ml of the resulting suspension were taken and mixed with the suspension of microconidiae. The homogenate was inactivated by adding thiomersal in a ratio of 1:20000 (w/v) directly to the cell suspension. For this purpose 50 mg of thiomersal were added to 1 litre of homogenate. The mixture was incubated at room temperature for 2 days.
The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection.

**Example 22**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9469, Trichophyton rubrum DSM-9471, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456, Candida albicans DSM-9457 were used to prepare 1.5 litres of the vaccine. Cultures of strains DSM-7279, 9469, 9471, 9472 were cultivated separately on malt extract agar in 4 Roux flasks for each culture for 20 days at 28°C. Cultures of Candida albicans strains DSM-9456, 9457 were cultivated in 1 Roux flask for each culture on agar Sabouraud at 28°C for 3 days.
The fungal masses of strains DSM-9469, 9471 and 9472 were lifted off, combined and homogenised in 100 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The fungal masses of strain DSM-7279 and the mixture of strains DSM-9469, 9471 and 9472 were lifted off and separately homogenised in 500 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The concentration of microconidia was adjusted to 60 million per ml of the homogenate. 500 ml of the Trichophyton mentagrophytes DSM-7279 suspension were mixed with 500 ml of the Trichophyton rubrum DSM-9469, 9471, 9472 mixture suspension in a single container.
The blastospores of strain DSM-9456, 9457 were lifted off by washing with 200 ml of destilled water. The concentration of blastospores in suspension was adjusted to 60 million per ml. 250 ml of each suspension were mixed.
500 ml of the resulting suspension were mixed with the suspension of microconidiae. The homogenate was inactivated by adding thiomersal in a ratio of 1:20000 (w/v) directly to the cell suspension. For this purpose 50 mg of thiomersal were added to 1 litre of homogenate. The mixture was incubated at room temperature for 2 days. The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection.

**Example 23**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456, Candida albicans DSM-9457, Candida albicans DSM-9459 were used to prepare 1.5 litres of the vaccine. Cultures of strains DSM-7279, 9472 were cultivated separately on malt extract agar in 6 Roux flasks for each culture for 20 days at 28°C. Cultures of Candida albicans strains DSM-9456, 9457, 9459 were cultivated in 1 Roux flask for each culture on agar Sabouraud at 28°C for 3 days.
The fungal masses of strains DSM-7279 and 9472 were lifted off and separately homogenised in 500 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The concentration of microconidia was adjusted to 60 million per ml of the homogenate. 500 ml of the Trichophyton mentagrophytes DSM-7279 suspension were mixed with 500 ml of the Trichophyton rubrum DSM-9472 suspension in a single container.
The blastospores of strain DSM-9456, 9457, 9459 were lifted off by washing with 200 ml of destilled water. The concentration of blastospores in suspension was adjusted to 60 million per ml. 250 ml of each suspension were mixed.
500 ml of the resulting suspension were mixed with the suspension of microconidiae. The homogenate was inactivated by adding thiomersal in a ratio of 1:20000 (w/v) directly to the cell suspension. For this purpose 50 mg of thiomersal were added to 1 litre of homogenate. The mixture was incubated at room temperature for 2 days. The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection.

**Example 24**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456 were used to prepare 1.5 litres of the vaccine. Trichophyton mentagrophytes DSM-7279 and Trichophyton rubrum DSM-9472 were cultivated separately on malt extract agar in 6 Roux flasks for each culture for 20

days at 28°C. The Candida albicans DSM-9456 was cultivated in 2 Roux flasks on agar Sabouraud at 28°C for 3 days. The fungal masses of the strains DSM-7279 and 9472 were lifted off and separately homogenised in 500 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The concentration of microconidia was adjusted to 60 million per ml of the homogenate. 500 ml of each suspension were mixed in a single container.

The blastospores of strain DSM-9456 were lifted off by washing with 500 ml of destilled water. The concentration of blastospores in suspension was adjusted to 10 million per ml.

500 ml of this suspension were mixed with the suspension of microconidiae.

The homogenate was inactivated by adding thiomersal in a ratio of 1:20000 (w/v) directly to the cell suspension. For this purpose 50 mg of thiomersal were added to 1 litre of homogenate. The mixture was incubated at room temperature for 2 days.

Following the inactivation the cell suspension was treated with sodium permanganate in a concentration of 1:20000 (w/v) for 36 hours while stirring. Treated cells were washed 5 times with destilled water by centrifugation (4000 rpm) for 25 minutes for each centrifugation step. The final concentration of the cells was adjusted to 60 million per ml.

The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection.

**Example 25**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9469, Trichophyton rubrum DSM-9470, Trichophyton rubrum DSM-9471, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456, Candida albicans DSM-9457, Candida albicans DSM-9458, Candida albicans DSM-9459 were used to prepare 1.5 litres of the vaccine. Cultures of strains DSM-7279, 9469, 9470, 9471, 9472 were cultivated separately on malt extract agar in 3 Roux flasks for each culture for 20 days at 28°C. Cultures of Candida albicans strains DSM-9456, 9457, 9458, 9459 were cultivated in 1 Roux flask for each culture on agar Sabouraud at 28°C for 3 days.

The fungal masses of strains DSM-9469, 9470, 9471 and 9472 were lifted off, combined and homogenised in 100 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The fungal masses of strain DSM-7279 and the mixture of strains DSM-9469, 9470, 9471 and 9472 were lifted off and separately homogenised in 500 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The concentration of microconidia was adjusted to 60 million per ml of the homogenate. 500 ml of the Trichophyton mentagrophytes DSM-7279 suspension were mixed with 500 ml of the Trichophyton rubrum DSM-9469, 9470, 9471, 9472 mixture suspension in a single container.

The blastospores of strain DSM-9456, 9457, 9458, 9459 were lifted off by washing with 200 ml of destilled water. The concentration of blastospores in suspension was adjusted to 60 million per ml. 150 ml of each suspension were mixed. 500 ml of the resulting suspension were mixed with the suspension of microconidiae. The homogenate was inactivated by adding thiomersal in a ratio of 1:20000 (w/v) directly to the cell suspension. For this purpose 50 mg of thiomersal were added to 1 litre of homogenate. The mixture was incubated at room temperature for 2 days.

Following the inactivation the cell suspension was treated with $H_2O_2$.

Hydrogen peroxide tablets (Wasserstoff-Peroxid Tabletten WDT) were added to cell suspensions to yield a final concentration of 3% of $H_2O_2$. The cell suspension was stirred for 24 hours. Treated cells were washed 5 times for 25 minutes with destilled water by centrifugation (4000 rpm). The final concentration of cells was adjusted to 80 million per ml.

The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection.

**Example 26**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9469, Trichophyton rubrum DSM-9471, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456, Candida albicans DSM-9457 were used to prepare 1.5 litres of the vaccine. Cultures of strains DSM-7279, 9469, 9471, 9472 were cultivated separately on malt extract agar in 4 Roux flasks for each culture for 20 days at 28°C. Cultures of Candida albicans strains DSM-9456, 9457 were cultivated in 1 Roux flask for each culture on agar Sabouraud at 28°C for 3 days.

The fungal masses of strains DSM-9469, 9471 and 9472 were lifted off, combined and homogenised in 100 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The fungal masses of strain DSM-7279 and the mixture of strains DSM-9469, 9471 and 9472 were lifted off and separately homogenised in 500 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The concentration of microconidia was adjusted to 60 million per ml of the homogenate. 500 ml of the Tricho-

phyton mentagrophytes DSM-7279 suspension were mixed with 500 ml of the Trichophyton rubrum DSM-9469, 9471, 9472 mixture suspension in a single container.
The blastospores of strain DSM-9456, 9457 were lifted off by washing with 200 ml of destilled water. The concentration of blastospores in suspension was adjusted to 60 million per ml. 250 ml of each suspension were mixed.
500 ml of the resulting suspension were mixed with the suspension of microconidiae. The homogenate was inactivated by adding thiomersal in a ratio of 1:20000 (w/v) directly to the cell suspension. For this purpose 50 mg of thiomersal were added to 1 litre of homogenate. The mixture was incubated at room temperature for 2 days.
Following the inactivation the cell suspension was treated with sodium permanganate in a concentration of 1:10000 (w/v) for 36 hours while stirring. Treated cells were washed 5 times with destilled water by centrifugation (4000 rpm) for 25 minutes for each centrifugation step. The final concentration of cells was adjusted to 60 million per ml.
The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection.

**Example 27**

Cultures of the strains Trichophyton mentagrophytes DSM-7279, Trichophyton rubrum DSM-9472 and Candida albicans DSM-9456, Candida albicans DSM-9457, Candida albicans DSM-9459 were used to prepare 1.5 litres of the vaccine. Cultures of strains DSM-7279, 9472 were cultivated separately on malt extract agar in 6 Roux flasks for each culture for 20 days at 28°C. Cultures of Candida albicans strains DSM-9456, 9457, 9459 were cultivated in 1 Roux flask for each culture on agar Sabouraud at 28°C for 3 days.
The fungal masses of strains DSM-7279 and 9472 were lifted off and separately homogenised in 500 ml of an aqueous solution of 0.3% fermented hydrolyzed muscle protein, 5% glucose and 0.1% yeast extract. The concentration of microconidia was adjusted to 60 million per ml of the homogenate. 500 ml of the Trichophyton mentagrophytes DSM-7279 suspension were mixed with 500 ml of the Trichophyton rubrum DSM-9472 suspension in a single container.
The blastospores of strain DSM-9456, 9457, 9459 were lifted off by washing with 200 ml of destilled water. The concentration of blastospores in suspension was adjusted to 60 million per ml. 250 ml of each suspension were and mixed. 500 ml of the resulting suspension were mixed with the suspension of microconidiae. The homogenate was inactivated by adding thiomersal in a ratio of 1:20000 (w/v) directly to the cell suspension. For this purpose 50 mg of thiomersal were added to 1 litre of homogenate. The mixture was incubated at room temperature for 2 days.
Following the inactivation the cell suspension was treated with $H_2O_2$. A substance containing $H_2O_2$, for example Urea-hydrogen peroxide, was added to the cell suspension to yield a final concentration of 3% of $H_2O_2$. The cell suspension was stirred for 36 hours. Treated cells were washed 5 times for 25 minutes with destilled water by centrifugation (4000 rpm). The final concentration of the cells was adjusted to 60 million per ml.
The resulting vaccine was bottled, checked for sterility, safety and immunogenic properties in accordance with accepted methods and kept refrigerated at 4°C. The vaccine prepared in this manner was used to immunise animals by intramuscular injection.

**Example 28**

**Efficacy of the vaccines after $LD_{50}$ Candida albicans challenge in mice**

The challenge was applied through intraperitoneal injection of 45 million Candida albicans blastospores per mouse. A single dose of 0.3 ml of the vaccine was applied subcutaneously on the same day as the challenge and a second dose after 7 days. The observation was continued for 4 weeks after the initial injection of vaccine.
Complexes 1-I: 1-II, 2-I were tested in this manner (see tables 1, 2, 3, 4).

**Example 29**

**Efficacy of the vaccines after $ID_{100}$ Candida albicans challenge in mice**

The challenge was applied through intraperitoneal injection of 10 million Candida albicans blastospores per mouse. A single dose of 0.3 ml of the vaccine was applied subcutaneously on the same day as the challenge and a second dose after 7 days. The observation was continued for 4 weeks after the initial injection of vaccine. Complexes 3-I; 3-II, 4-I were tested in this manner (see tables 5 and 6).

**Example 30**

**Efficacy of the vaccines after Trichophyton challenge in guinea pigs**

The challenge of Trichophyton rubrum microconidiae consisted of 500 thousand microconidia per $cm^2$ (1.5 million microconidia) applied topically for each animal.
The challenge of Trichophyton mentagrophytes microconidia consisted of 100-200 thousand microconidia per $cm^2$ (300-600 thousand microconidia) applied topically for each animal.
A single dose of 1.0 ml of the vaccine was applied through intramuscular injection on the same day as the challenge and a second dose after 7 days. The observation was continued for 4 weeks after the initial injection of vaccine. Complexes I-I, I-II, II-I, II-II, III-I, III-II, III-III, III-IV, III-V (see tables 7, 8, 9, 10, 11, 12, 13, 14 and figures 1-18) were tested.
The clinical symptoms of a Trichophyton infection in guinea pigs were evaluated using the following severity scores:

0 = no symptoms
1 = hyperaemia of the skin at the place of fungi application
2 = single spots of scaling
3 = scaling of the skin at the place of fungi application
4 = thin small crusts at the place of fungi application
5 = scab-like crusts at the place of fungi application

**Example 31**

**Efficacy of the vaccines after Trichophyton challenge in rabbits**

The challenge of Trichophyton rubrum microconidiae consisted of 500 thousand microconidia per $cm^2$ (1.5 million microconidia) applied topically for each animal.
A single dose of 2.0 ml of the vaccine was applied through by intramuscular injection on the same day as the challenge and a second dose after 7 days.
The observation was continued for 4 weeks after the initial injection of vaccine. Complex II-I (see tables 15, 16 and figures 19, 20) was tested. The clinical symptoms of a Trichophyton infection in rabbits were evaluated using the same severity scores cited in example 30.

**Results of an $LD_{50}$ Candida albicans challenge in vaccinated mice (1st experiment)**

Table 1

| **Acute pathogenic activity** (For method see example 28) | | | |
|---|---|---|---|
| COMPLEXES | Number of animals | Number of died animals during acute period | % loss of animals |
| Complex 1-I (Example 8) | 10 | 5 | 50 |
| Complex 1-II (Example 16) | 10 | 4 | 40 |
| Control (destilled water) | 11 | 5 | 45.5 |

When using the $LD_{50}$ challenge dose there was the same death rate in mice (40-50%) in experimental and control groups of animals during the period of acute pathogenicity (3 days after injection).

Table 2

| **Development of the disease** (For method see example 28) | | | |
|---|---|---|---|
| COMPLEXES | Number of animals | Number of animals with symptoms of Candida albicans infection | % of infected animals |
| Complex 1-I (Example 8) | 5 | 2 | 40 |
| Complex 1-II (Example 16) | 6 | 2 | 33.3 |
| Control (destilled water) | 6 | 6 | 100 |

During the follow-up period (day 4 to day 28) 100% of the survivors in the unvaccinated control group developed clinical symptoms of candidiasis while the efficacy rate in vaccinated animals was 60% (Complex 1-I) and 66.7% (Complex 1-II) respectively.

**Results of an $LD_{50}$ Candida albicans challenge in vaccinated mice (2nd experiment)**

Table 3

| **Acute pathogenic activity** (For method see example 28) | | | |
|---|---|---|---|
| COMPLEXES | Number of animals | Number of loss of mice during acute period | % loss of mice |
| Complex 2-I (Example 16) | 10 | 4 | 40 |
| Untreated control | 11 | 4 | 36 |

Using the $LD_{50}$ challenge dose 40% and 36 % of the animals died in the experimental group and control group respectively during the period of acute pathogenicity (3 days after injection).

Table 4

| **Development of the disease** (For method see example 28) | | | |
|---|---|---|---|
| COMPLEXES | Number of animals | Number of animals with symptoms of Candida albicans infection | % of infected animals |
| Complex 2-I (Example 16) | 6 | 3 | 50 |
| Untreated control | 7 | 7 | 100 |

During the follow up period (day 4 to day 28) 100% of the survivors in the unvaccinated control group developed clinical symptoms of candidiasis while the efficacy rate in vaccinated animals was 50% (Complex 2-I).

**Results of an $ID_{100}$ Candida albicans challenge in vaccinated mice (3rd experiment)**

Table 5

| **Development of the disease** (For method see example 29) | | | |
|---|---|---|---|
| COMPLEXES | Number of animals | Number of animals with symptoms of Candida albicans infection | % of infected animals |
| Complex 3- I (Example 8) | 10 | 3 | 30 |
| Complex 3-II (Example 16) | 10 | 7 | 70 |
| Untreated control | 11 | 9 | 82 |

When using $ID_{100}$ 70% of the animals vaccinated by Complex 3-I and 30% of the animals vaccinated by Complex 3-II were healthy while 82% of the animals in the control group suffered from clinical symptoms of candidiasis.

**Results of an $ID_{100}$ Candida albicans challenge in vaccinated mice (4th experiment)**

Table 6

| **Development of disease** (For method see example 29) | | | |
|---|---|---|---|
| COMPLEXES | Number of animals | Number of animals with symptoms of Candida albicans infection | % of infected animals |
| Complex 4-I (Example 12) | 10 | 1 | 10 |
| Untreated control | 10 | 8 | 80 |

When using $ID_{100}$ 90% of the animals vaccinated by Complex 4-I were healthy while 80% of the mice in the control group had clinical syptoms of candidiasis.

**Clinical symptoms of Trichophyton rubrum disease in guinea pigs (1st experiment)**

Table 7

| (For method see example 30) | | | | | |
|---|---|---|---|---|---|
| | | Date of observation | | | |
| Complexes | animal No. | day 7 | day 13 | day 21 | day 28 |
| Complex I-I (Example 8) | 1/1 | 0 | 4 | 3 | 0 |
| | 1/2 | 0 | 2 | 2 | 0 |
| | 1/3 | 0 | 4 | 2 | 0 |
| | 2/1 | 0 | 4 | 3 | 0 |
| | 2/2 | 0 | 2 | 2 | 0 |
| | mean | 0 | 3.2 | 2.4 | 0 |
| Complex I-II (Example 16) | 3/1 | 0 | 3 | 3 | 0 |
| | 3/2 | 0 | 2 | 2 | 2 |
| | 3/3 | 1 | 4 | 3 | 0 |
| | 4/1 | 1 | 2 | 3 | 2 |
| | 4/2 | 1 | 3 | 3 | 0 |
| | mean | 0.6 | 2.8 | 2.8 | 0.8 |
| Untreated control | 11 | 1 | 5 | 4 | 3 |
| | 12 | 1 | 5 | 4 | 2 |
| | 13 | 1 | 5 | 4 | 2 |
| | 14 | 1 | 5 | 4 | 0 |
| | 15 | 1 | 5 | 4 | 2 |
| | mean | 1 | 5 | 4 | 1.8 |

The severity of clinical symptoms of rubrophytosis in challenged guinea pigs is shown after different observation periods. Compared with vaccinated animals (Complexes I-I and I-II) unvaccinated control animals had more severe clinical symptoms (see figs. 1, 2, 3).

**Number of guinea pigs with clinical symptoms of Trichophyton rubrum disease (1st experiment)**

Table 8

| (For method see example 30) | | | | |
|---|---|---|---|---|
| | Date of observation | | | |
| COMPLEXES | day 7 | day 13 | day 21 | day 28 |
| Complex I-I (Example 8) | 0/5 | 5/5 | 5/5 | 0/5 |
| Complex I-II (Example 16) | 3/5 | 5/5 | 5/5 | 2/5 |
| Untreated control | 5/5 | 5/5 | 5/5 | 4/5 |
| (Note: number of animals with clinical symptoms / number of challenged animals) | | | | |

Compared with the control group there were less animals with clinical symptoms on day 7 and 28 after vaccination

(see figs. 1, 2, 3)

**Clinical symptoms of Trichophyton rubrum disease in guinea pigs (2nd experiment)**

Table 9

| (For method see example 30) | | | | | |
|---|---|---|---|---|---|
| | | Date of observation | | | |
| Complexes | animal No. | day 7 | day 15 | day 21 | day 28 |
| Complex II-I (Example 1) | 2 | 0 | 2 | 2 | 2 |
| | 3 | 0 | 3 | 0 | 0 |
| | 4 | 0 | 2 | 0 | 0 |
| | mean | 0 | 2.3 | 0.66 | 0.66 |
| Complex II-II (Example 20) | 1 | 0 | 2 | 2 | 2 |
| | 2 | 2 | 2 | 2 | 2 |
| | 3 | 0 | 2 | 0 | 0 |
| | 4 | 0 | 3 | 2 | 2 |
| | mean | 0.5 | 2.25 | 1.5 | 1.5 |
| Untreated control | 1 | 3 | 4 | 3 | 3 |
| | 2 | 3 | 5 | 5 | 5 |
| | 3 | 4 | 3 | 2 | 2 |
| | 4 | 4 | 3 | 3 | 1 |
| | 5 | 2 | 2 | 3 | 2 |
| | mean | 3.2 | 3.4 | 3.2 | 2.6 |

The severity of clinical symptoms of rubrophytosis in challenged guinea pigs is shown after different observation periods. Compared with vaccinated animals (Complexes II-I and II-II) unvaccinated control animals had more severe clinical symptoms (see figs. 4, 5, 6).

**Number of guinea pigs with clinical symptoms of Trichophyton rubrum disease (2nd experiment)**

Table 10

| (For method see example 30) | | | | |
|---|---|---|---|---|
| | Date of observation | | | |
| COMPLEXES | day 7 | day 15 | day 21 | day 28 |
| Complex II-I (Example 1) | 0/3 | 3/3 | 1/3 | 1/3 |
| Complex II-II (Example 20) | 1/4 | 4/4 | 3/4 | 3/4 |
| Untreated control | 5/5 | 5/5 | 5/5 | 5/5 |
| (Note: number of animals with clinical symptoms / number of challenged animals) | | | | |

Compared with the control group there were less animals with clinical symptoms in both vaccination groups at each day of observation (see figs. 4, 5, 6)

**Clinical symptoms of Trichophyton rubrum disease in guinea pigs (3rd experiment)**

Table 11

(For method see example 30)

| | | Date of observation | | | |
|---|---|---|---|---|---|
| Complexes | animal No. | day 7 | day 16 | day 21 | day 28 |
| Complex III-I (Example 1) | 1 | 1 | 2 | 0 | 0 |
| | 2 | 0 | 2 | 0 | 0 |
| | 3 | 1 | 4 | 0 | 0 |
| | 4 | 0 | 2 | 0 | 0 |
| | 5 | 1 | 3 | 0 | 0 |
| | mean | 0.6 | 2.6 | 0 | 0 |
| Complex III-II (Example 2) | 2 | 1 | 2 | 0 | 0 |
| | 3 | 1 | 2 | 0 | 0 |
| | 4 | 1 | 4 | 0 | 0 |
| | 5 | 0 | 2 | 0 | 0 |
| | mean | 0.75 | 2.5 | 0 | 0 |
| Complex III-III (Example 3) | 1 | 1 | 4 | 2 | 2 |
| | 2 | 2 | 4 | 2 | 2 |
| | 3 | 2 | 4 | 2 | 2 |
| | 5 | 2 | 4 | 2 | 2 |
| | mean | 1.75 | 4 | 2 | 2 |
| Complex III-IV (Example 4) | 1 | 3 | 4 | 2 | 1 |
| | 2 | 3 | 4 | 3 | 1 |
| | 3 | 1 | 4 | 2 | 1 |
| | 5 | 3 | 4 | 2 | 2 |
| | mean | 2.5 | 4 | 2.25 | 1.25 |
| Complex III-V (Example 12) | 1 | 2 | 4 | 3 | 2 |
| | 2 | 2 | 4 | 2 | 2 |
| | 3 | 2 | 2 | 2 | 1 |
| | 4 | 1 | 2 | 3 | 1 |
| | 5 | 2 | 4 | 3 | 2 |
| | mean | 1.8 | 3.2 | 2.6 | 1.6 |
| Untreated control | 1 | 3 | 5 | 4 | 3 |
| | 2 | 3 | 5 | 4 | 1 |
| | 4 | 2 | 5 | 4 | 1 |
| | 5 | 3 | 5 | 4 | 2 |
| | mean | 2.75 | 5 | 4 | 1.75 |

The severity of clinical symptoms of rubrophytosis in challenged guinea pigs is shown after different observation periods. Compared with vaccinated animals (Complexes III-I, III-II, III-II, III-IV, III-V) unvaccinated control animals had

more severe clinical symptoms (see figs. 7, 8, 9, 10, 11, 12).

**Number of guinea pigs with clinical symptoms of Trichophyton rubrum disease (3rd experiment)**

Table 12

| (For method see example 30) | | | | |
|---|---|---|---|---|
| | Date of observation | | | |
| COMPLEXES | day 7 | day 16 | day 21 | day 28 |
| Complex III-I (Example 1) | 3/5 | 5/5 | 0/5 | 0/5 |
| Complex III-II (Example 2) | 3/4 | 4/4 | 0/4 | 0/4 |
| Complex III-III (Example 3) | 4/4 | 4/4 | 4/4 | 4/4 |
| Complex III-IV (Example 4) | 4/4 | 4/4 | 4/4 | 4/4 |
| Complex III-V (Example 12) | 5/5 | 5/5 | 5/5 | 5/5 |
| Untreated control | 4/4 | 4/4 | 4/4 | 4/4 |
| (Note: number of animals with clinical symptoms / number of challenged animals) | | | | |

**Clinical symptoms of Trichophyton mentagrophytes disease in guinea pigs (3rd experiment)**

Table 13

(For method see example 30)

| Complexes | animal No. | Date of observation | | | |
|---|---|---|---|---|---|
| | | day 7 | day 16 | day 21 | day 28 |
| Complex III-I (Example 1) | 2 | 2 | 4 | 2 | 1 |
| | 3 | 2 | 4 | 3 | 1 |
| | 4 | 2 | 4 | 2 | 1 |
| | 5 | 2 | 4 | 2 | 1 |
| | mean | 2 | 4 | 2.25 | 1 |
| Complex III-II (Example 2) | 1 | 1 | 4 | 4 | 0 |
| | 2 | 2 | 5 | 2 | 1 |
| | 3 | 2 | 4 | 2 | 1 |
| | 4 | 2 | 4 | 2 | 1 |
| | 5 | 2 | 2 | 2 | 1 |
| | mean | 1.8 | 3.8 | 2.4 | 0.8 |
| Complex III-III (Example 3) | 1 | 4 | 5 | 1 | 0 |
| | 2 | 4 | 4 | 1 | 0 |
| | 3 | 4 | 5 | 2 | 2 |
| | 4 | 4 | 4 | 2 | 2 |
| | 5 | 4 | 5 | 2 | 2 |
| | mean | 4 | 4.6 | 1.6 | 1.2 |
| Complex III-IV (Example 4) | 1 | 4 | 5 | 2 | 0 |
| | 3 | 3 | 4 | 2 | 1 |
| | 5 | 4 | 4 | 2 | 1 |
| | mean | 3.6 | 4.3 | 2 | 0.6 |
| Complex III-V (Example 12) | 1 | 2 | 5 | 2 | 1 |
| | 2 | 4 | 5 | 3 | 1 |
| | 3 | 4 | 5 | 3 | 1 |
| | 4 | 4 | 5 | 2 | 2 |
| | 5 | 4 | 4 | 2 | 2 |
| | mean | 3.6 | 4.8 | 2.4 | 1.4 |
| Untreated control | 1 | 4 | 4 | 2 | 1 |
| | 2 | 4 | 5 | 3 | 1 |
| | 3 | 4 | 4 | 5 | 4 |
| | 4 | 4 | 4 | 3 | 3 |
| | 5 | 4 | 5 | 5 | 4 |
| | mean | 4 | 4.4 | 3.6 | 2.6 |

The severity of clinical symptoms of Trichophyton mentagrophytes infection in challenged guinea pigs is shown

after different observation periods. Compared with vaccinated animals (Complexes III-I, III-II, III-III, III-IV, III-V) unvaccinated control animals had more severe clinical symptoms
(see figs. 13, 14, 15, 16, 17, 18).

**Number of guinea pigs with clinical symptoms of Trichophyton mentagrophytes disease (3rd experiment)**

Table 14

| (For method see example 30) | | | | |
|---|---|---|---|---|
| | Date of observation | | | |
| COMPLEX | day 7 | day 16 | day 21 | day 28 |
| Complex III-I (Example 1) | 4/4 | 4/4 | 4/4 | 4/4 |
| Complex III-II (Example 2) | 5/5 | 5/5 | 5/5 | 4/5 |
| Complex III-III (Example 3) | 5/5 | 5/5 | 5/5 | 3/5 |
| Complex III-IV (Example 4) | 3/3 | 3/3 | 3/3 | 2/3 |
| Complex III-V (Example 12) | 5/5 | 5/5 | 5/5 | 2/5 |
| Untreated control | 5/5 | 5/5 | 5/5 | 5/5 |
| (Note: number of animals with clinical symptoms / number of challenged animals) | | | | |

Nearly all vaccinated animals displayed clinical symptoms during the observation period (see figs. 13, 14, 15, 16, 17, 18).

**Clinical symptoms of Trichophyton rubrum disease in rabbits (1st experiment)**

Table 15

| (For method see example 31) | | | | | |
|---|---|---|---|---|---|
| | | Date of observation | | | |
| Complex | animal No. | day 7 | day 15 | day 21 | day 28 |
| Complex II-I (Example 1) | 1 | 0 | 1 | 0 | 0 |
| | 2 | 1 | 3 | 0 | 0 |
| | 3 | 2 | 1 | 2 | 0 |
| | 4 | 2 | 2 | 0 | 0 |
| | 5 | 2 | 3 | 0 | 0 |
| | mean | 1.4 | 2 | 0.4 | 0 |
| Untreated control | 1 | 4 | 3 | 0 | 0 |
| | 2 | 1 | 3 | 3 | 3 |
| | 3 | 2 | 3 | 2 | 2 |
| | 4 | 4 | 5 | 3 | 3 |
| | 5 | 4 | 5 | 5 | 4 |
| | mean | 3 | 3.8 | 2.6 | 2.4 |

The severity of clinical symptoms of Trichophyton rubrum infection in challenged rabbits is shown after different observation periods. Compared with vaccinated animals (Complex II-I) unvaccinated control animals had more severe clinical symptoms

(see figs. 19, 20).

**Number of rabbits with clinical symptoms of Trichophyton rubrum disease (1st experiment)**

Table 16

| (For method see example 31) | | | | |
|---|---|---|---|---|
| | Date of observation | | | |
| COMPLEX | day 7 | day 15 | day 21 | day 28 |
| Complex II-I (Example 1) | 4/5 | 5/5 | 1/5 | 0/5 |
| Untreated control | 5/5 | 5/5 | 4/5 | 4/5 |
| (Note: number of animals with clinical symptoms / number of challenged animals) | | | | |

Compared with the control group nearly all vaccinated animals had no clinical symptoms on day 21 and 28 (see figs. 19, 20)

**Claims**

1. Vaccine comprising homogenised inactivated dermatophyte microconidia and inactivated yeast blastospores or antigenic material thereof.

2. Vaccine according to claim 1, characterised in that the blastospores are in a swollen condition and/or have germ tubes and/or the microconidia are in a swollen condition and/or have germ tubes.

3. Vaccine according to claim 1 or 2, characterised in that at least 50% of the blastospores are in a swollen condition and/or have germ tubes and/or at least 50% of the microconidia are in a swollen condition and/or have germ tubes

4. Vaccine according to any one of claims 1 to 3, characterised in that the yeast blastospores belong to the genus Candida and the dermatophyte microconidia belong to the genera Trichophyton and/or Microsporum.

5. Vaccine according to any one of claims 1 to 4, characterised in that the yeast blastospores belong to the species Candida albicans and the dermatophyte microconidia belong to the species Trichophyton rubrum and/or Trichophyton mentagrophytes and/or Microsporum canis.

6. Vaccine according to any one of claims 1 to 5, characterised in that the yeast blastospores belong to the strains Candida albicans DSM - 9456, and/or Candida albicans DSM - 9457 and/or Candida albicans DSM - 9458 and/or Candida albicans DSM - 9459 and dermatophyte microconidia belonging to the strains Trichophyton rubrum DSM - 9469 and/or Trichophyton rubrum DSM - 9470 and/or Trichophyton rubrum DSM - 9471 and/or Trichophyton rubrum DSM - 9472 and/or Trichophyton mentagrophytes DSM - 7279 and/ or Microsporum canis DSM - 7281.

7. Vaccine according to any one of claims 1 to 6, characterised in that the fungal spores have been inactivated with thiomersal, formaldehyde or 2-propiolactone.

8. Vaccine according to any one of claims 1 to 7, characterised in that the fungal spores have been modified after inactivation.

9. Vaccine according to any one of claims 1 to 8, characterised in that said fungal spores have been modified by treatment with $H_2O_2$ or salts of permanganate.

10. Vaccine according to any one of claims 1 to 9, characterised in that said Mycosis vaccine comprises no additional immunomodulatory substance.

11. Vaccine according to any one of claims 1 to 10, characterised in that said Mycosis vaccine comprises no adjuvant.

12. Vaccine according to any one of claims 1 to 11, characterised in that said Mycosis vaccine comprises an additional substance with immunomodulatory activity.

13. Vaccine according to any one of claims 1 to 8 or 12, characterised in that said Mycosis vaccine comprises an adjuvant and/or at least one cytokine.

14. Vaccine according to any one of claims 1 to 13, characterised in that it comprises 10 to 90 million spores per ml.

15. Vaccine according to any one of claims 1 to 14, characterised in that it comprises about 60 million spores per ml.

16. Use of a vaccine according to any one of claims 1 to 15 for the prophylaxis and treatment of mycoses.

17. Use of a vaccine according to any one of claims 1 to 16 for the prophylaxis and treatment of mycoses in humans.

18. Use of a vaccine according to any one of claims 1 to 17 for the prophylaxis and treatment of Dermatomycosis and/or Onychomycosis and/or Candidosis.

19. Use of a vaccine according to any one of claims 1 to 15 for modulating the immune response.

20. Use of a vaccine according to any one of claims 1 to 15 for stimulating the immune response.

21. Trichophyton rubrum strain no. DSM - 9469 and/or DSM - 9470 and/or DSM - 9471 and/or DSM - 9472.

22. Candida albicans strain no. DSM - 9456 and/or DSM - 9457 and/or DSM - 9458 and/or DSM - 9459.

23. Process for the preparation of a vaccine comprising:

A: growing a dermatophyte on suitable solid medium, harvesting and homogenising the dermatophyte,
B: growing a yeast on a suitable medium, harvesting and homogenising the yeast,
C: combining and inactivating the homogenates A and B.

24. Process for the preparation of a vaccine according to claim 23, characterised in that the dermatophyte is homogenised in aqueous solution comprising 0.1-0.3% fermented hydrolyzed muscle protein or 0.1-1% soy or pork peptone in combination with 5-6% glucose and 0.1-1% yeast extract and subsequently incubated for 1-2 days at 28°C.

25. Process for the preparation of a vaccine according to claim 23, characterised in that the yeast is incubated after homogenisation in the presence of 5-6% $CO_2$ for about 2 to 4 hours.

26. Process for the preparation of a vaccine according to any one of claims 23 to 25, characterised in that the fungi homogenates are treated with $H_2O_2$, or a permanganate salt.

27. Process for the preparation of an increased amount of swollen microconidia and microconidia with germ tubes of dermatophytes, comprising:

cultivating a dermatophyte on a solid medium,
harvesting and homogenising the culture in a liquid medium,
maintaining the pH of the liquid medium at 6.2 to 7.2,
transferring the suspension in a separate vessel containing fresh liquid medium,
monitoring the growth and morphological appearance of the dermatophyte cells,
harvesting the cells when no less then 50% of the microconidia display a swollen or germinating condition, and no more than 7-10% of the cells display a second mycelial branch.

28. Process according to claim 27, characterised in that the culture media are malt extract-agar or agar Sabouraud, and the liquid medium comprises 0.3-1.0% crude extract or peptone from meat or soya, comprising 5-6% glucose and 0.1-1.0% yeast extract or malt-extract broth or meat-glucose broth.

29. Process for the preparation of an increased amount of swollen blastospores and blastospores with germ tubes of yeast, comprising:

cultivating yeast on a solid medium,
harvesting and homogenising the yeast in a liquid medium,
incubating the homogenate in a $CO_2$ atmosphere of 5-6% at 36-38°C for 2-4 hours,
monitoring the growth and morphological appearance of the yeast cells,
harvesting the cells when no less then 50% of the blastospores display germ tubes or a swollen condition.

**30.** Process according to claim 29, characterised in that the liquid culture medium has a pH of 6.8-7.0.

**31.** Process according to any one of claims 23 to 30, characterised in that the dermatophyte belongs to the genus Trichophyton and/or Microsporum, and/or the species Trichophyton rubrum, Trichophyton mentagrophytes and/or Microsporum canis and/or the strain is Trichophyton rubrum DSM - 9469 and/or DSM - 9470 and/or DSM - 9471 and/or DSM - 9472 and/or Trichophyton mentagrophytes DSM - 7279 and/or Microsporum canis DSM - 7281 and the yeast is selected from the genus Candida or the species Candida albicans and/or the strains Candida albicans DSM - 9456 and/or DSM - 9457 and/or DSM - 9458 and/or DSM - 9459.

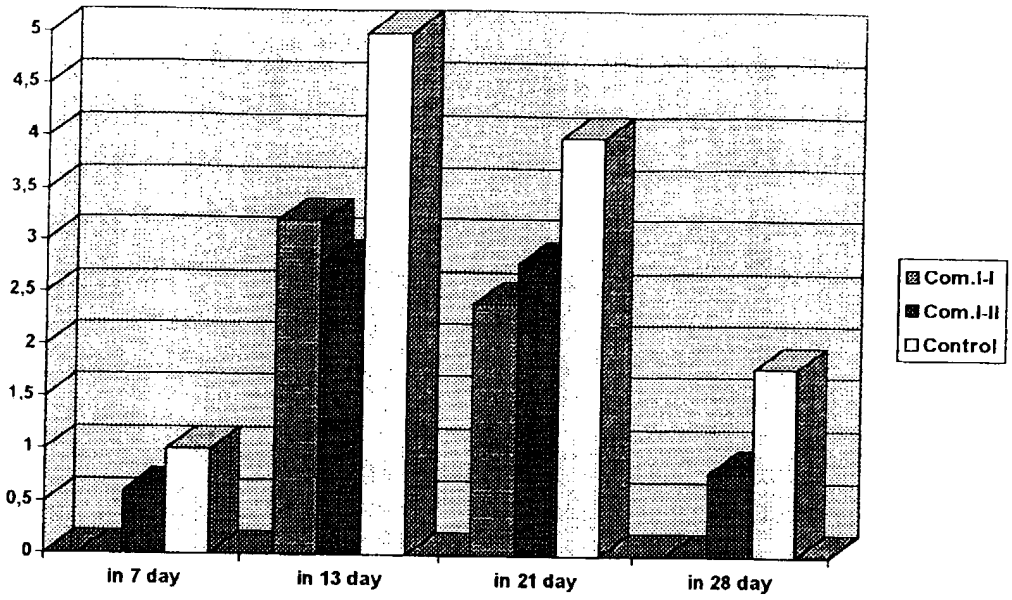


Figure 1

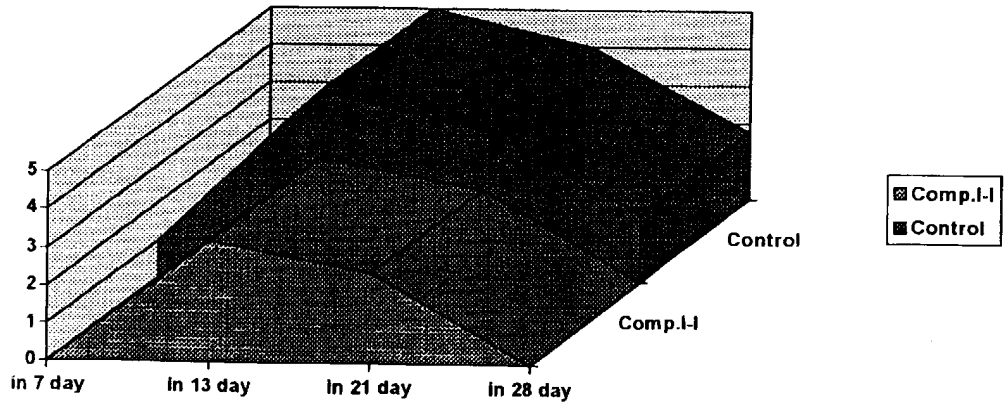


Figure 2

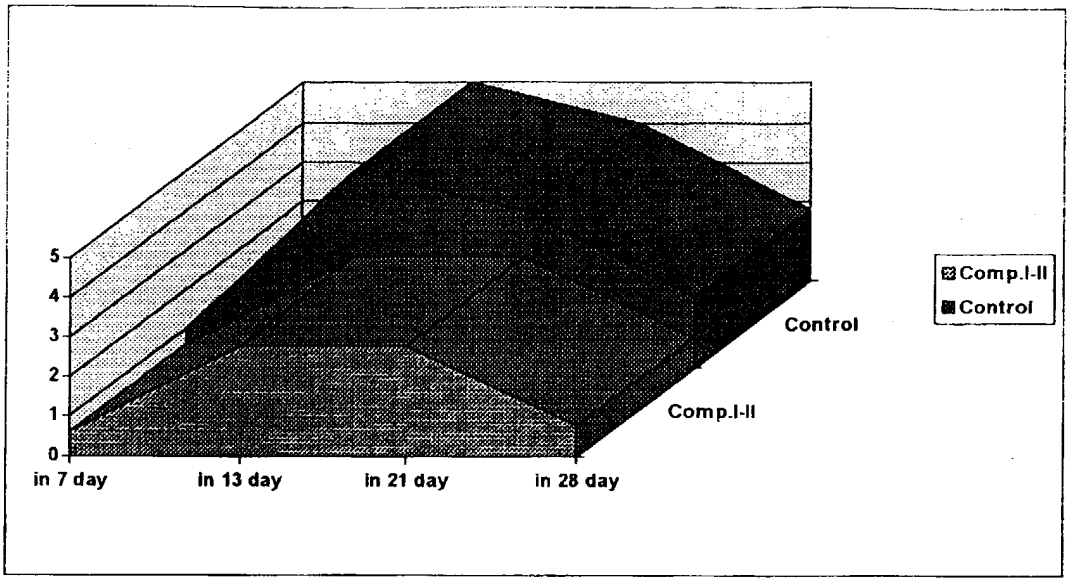


Figure 3

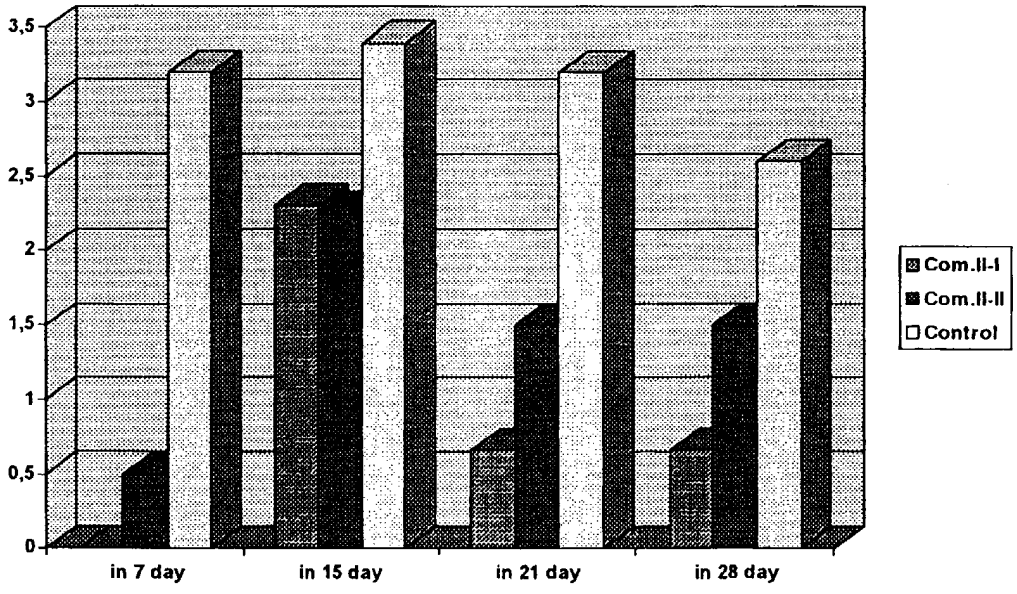


Figure 4

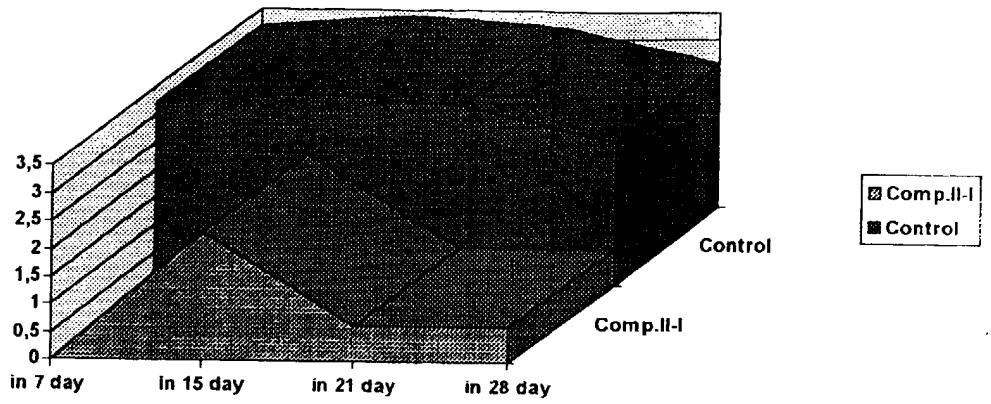


Figure 5

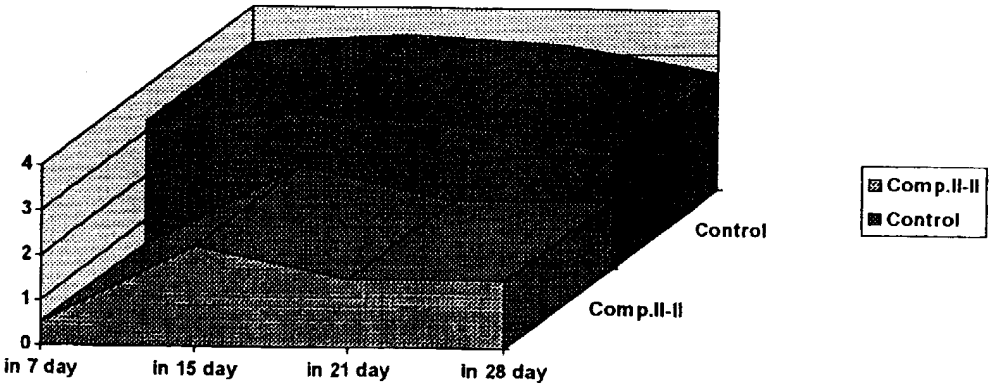


Figure 6

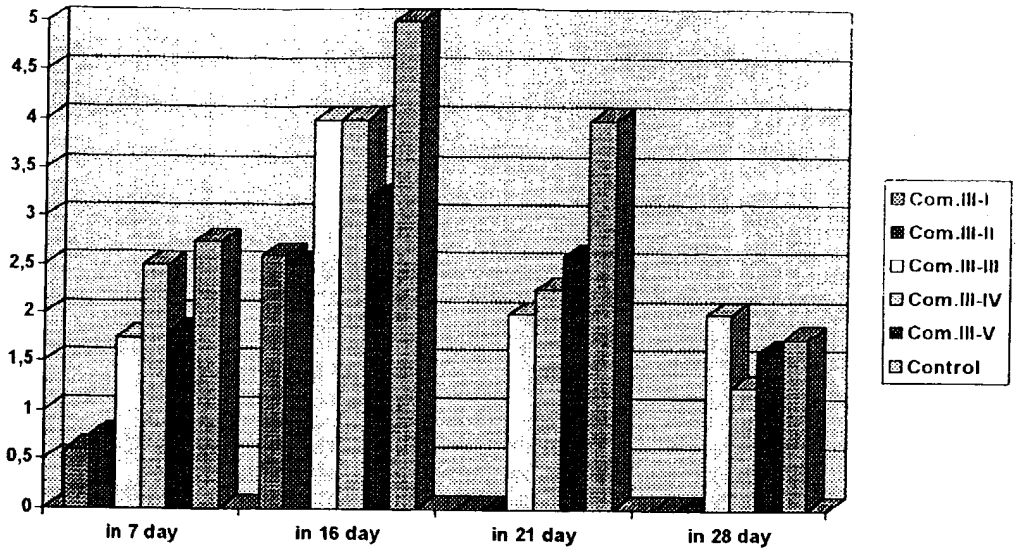


Figure 7

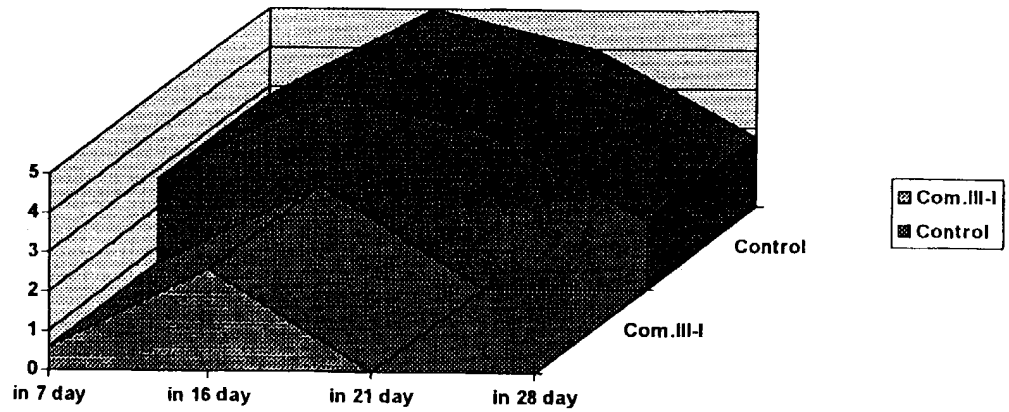


Figure 8

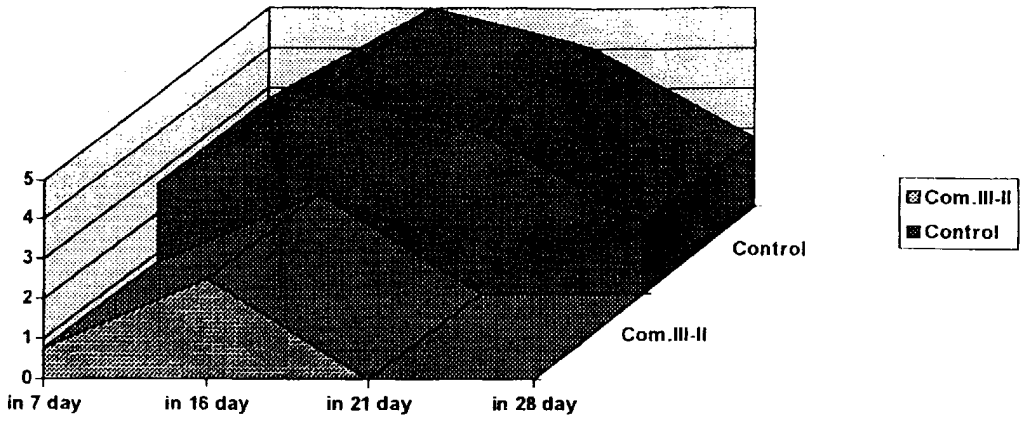


Figure 9

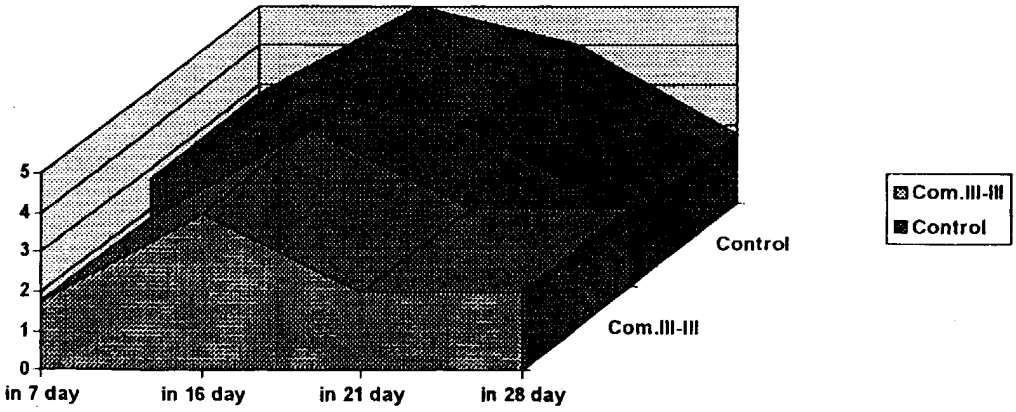


Figure 10

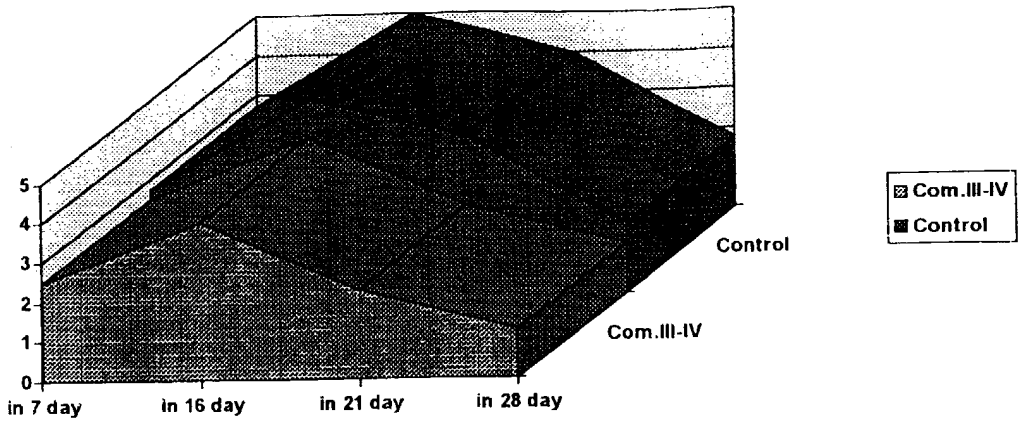


Figure 11

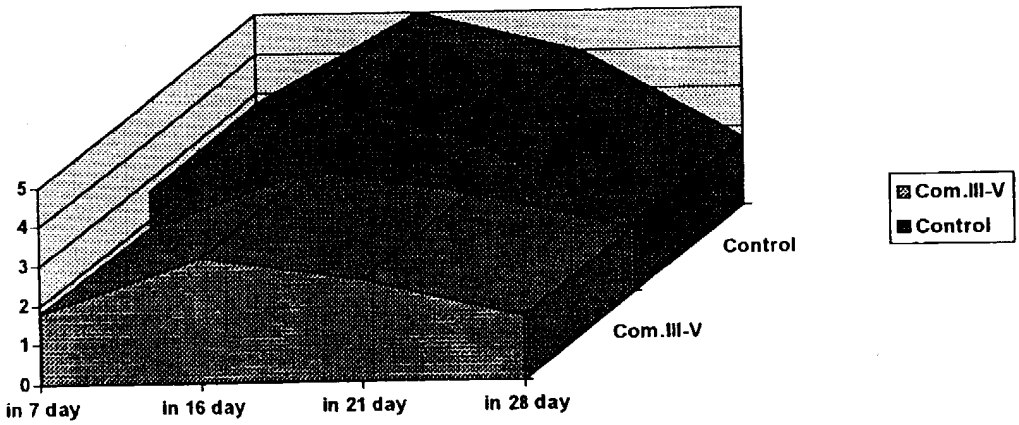


Figure 12

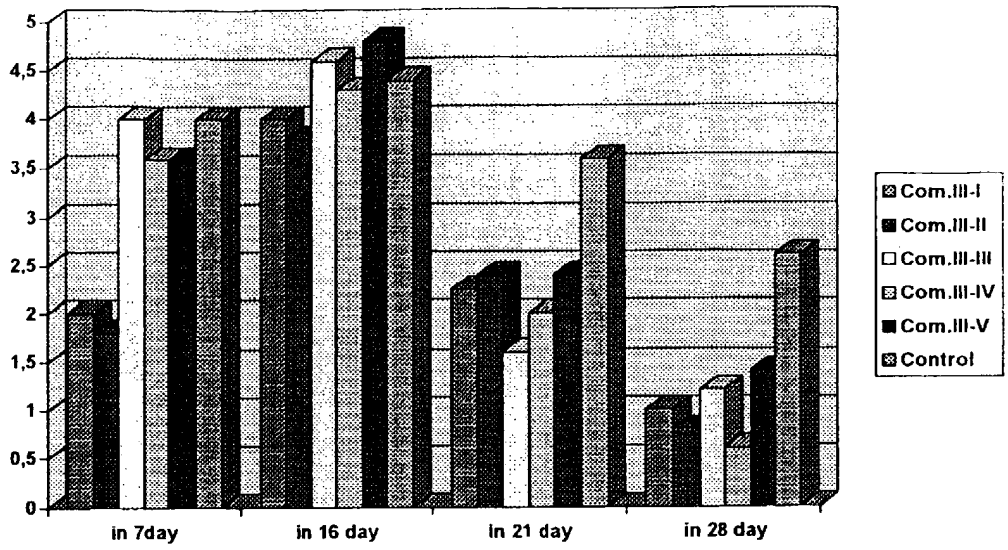


Figure 13

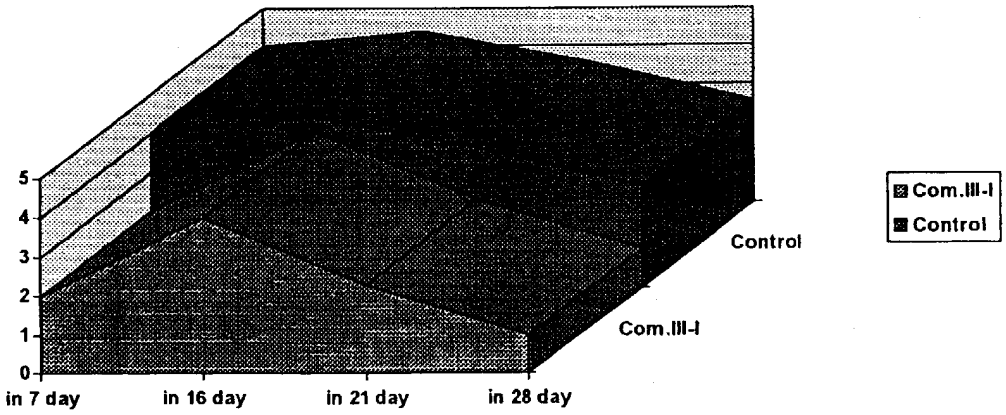


Figure 14

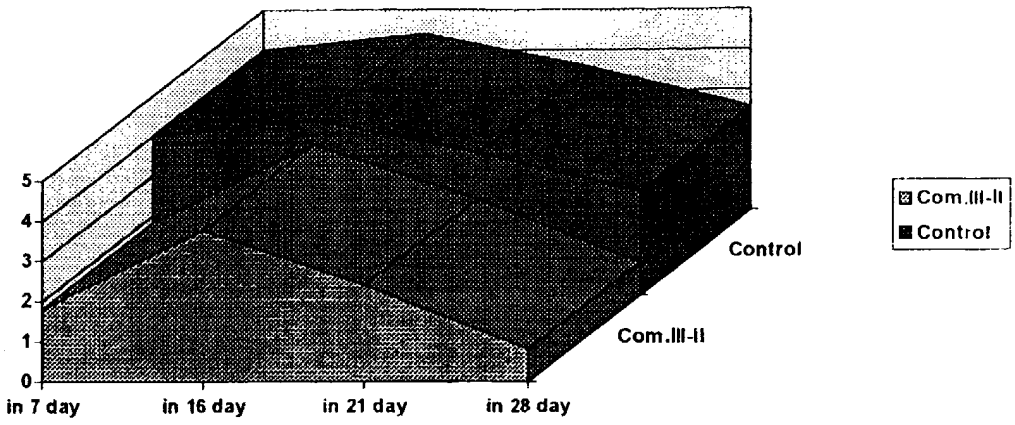


Figure 15

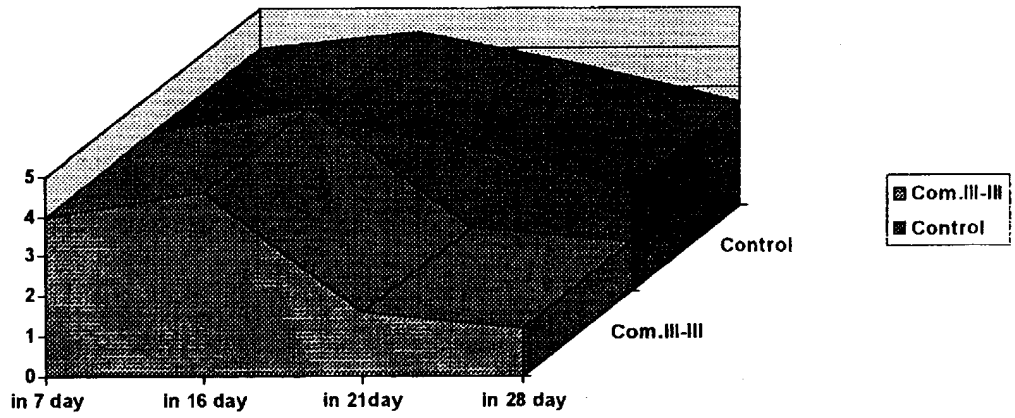


Figure 16

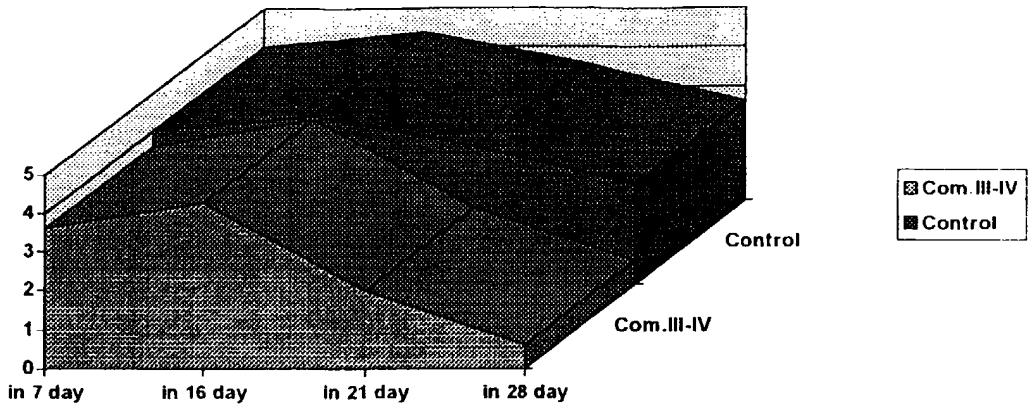


Figure 17

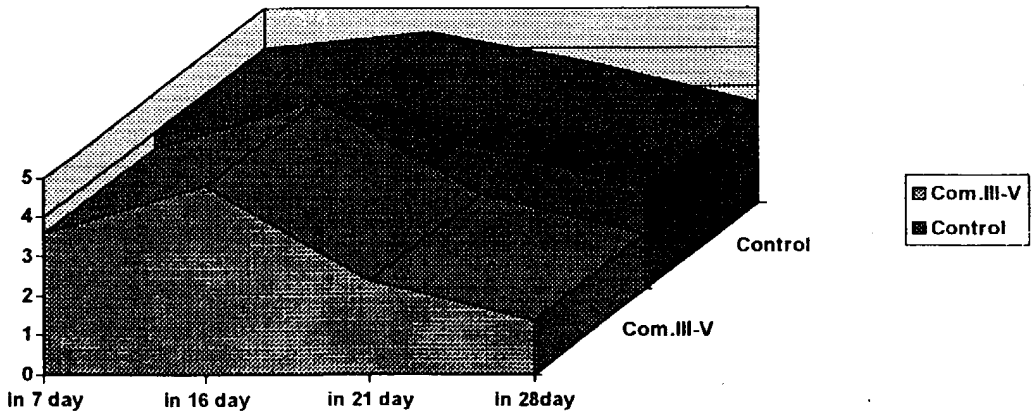


Figure 18

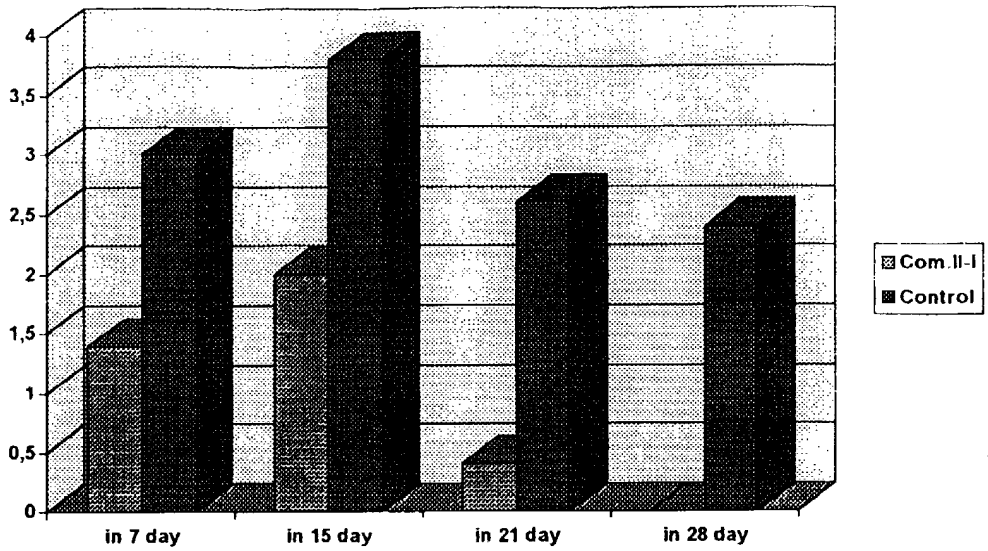


Figure 19

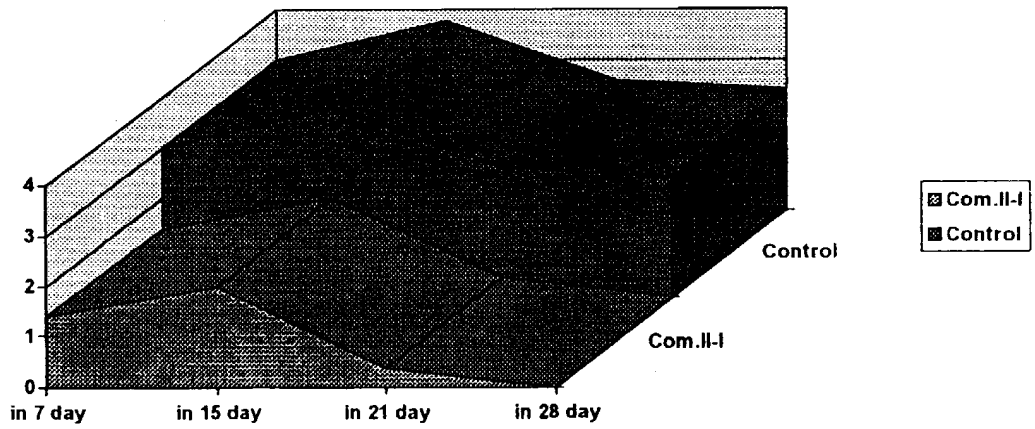


Figure 20